

# WO9805787

## Publication Title:

A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

## Abstract:

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The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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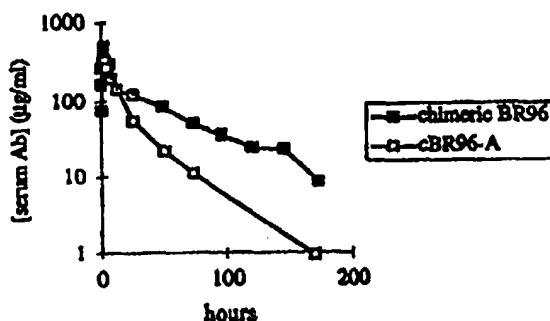
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International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C12N 15/62, A61K 39/395, 38/17, 47/48, 51/10, C07K 16/30, 16/46, 16/00, C12N 15/13, 1/21, 5/10 // C07K 19/00</b>		A1	(11) International Publication Number: <b>WO 98/05787</b> (43) International Publication Date: <b>12 February 1998 (12.02.98)</b>
(21) International Application Number: <b>PCT/US97/13562</b> (22) International Filing Date: <b>1 August 1997 (01.08.97)</b>		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(30) Priority Data: <b>60/023,033 2 August 1996 (02.08.96) US</b>		<b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
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(54) Title: **A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS**



Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

(57) Abstract

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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5 **A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED  
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THERAPY AND IN VIVO DIAGNOSIS**

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10 Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15 **TECHNICAL FIELD OF THE INVENTION**

The present invention relates to methods for inhibiting or reducing immunoglobulin-induced toxicity resulting from therapy or in vivo diagnosis. Specifically, in lieu of using unmodified antibodies or recombinant binding proteins for in vivo use, the 20 invention provides the use of modified antibodies or recombinant binding proteins which have been structurally altered in the constant domain so that upon administration immunoglobulin-induced toxicity is reduced or inhibited.

**BACKGROUND OF THE INVENTION**

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Over the years investigators have attempted to harness the immune system for therapeutic use. Immunoglobulin (Ig) molecules which constitute an important part of the immune system are of great interest because they (1) react with a diverse family of ligands, (2) possess different effector functions and (3) are of great 30 biological importance. Despite its potential, a persistent problem with

immunoglobulin immunotherapy has been, among other problems, the toxic effect to normal cells of using antibodies which recognize both normal and diseased cells. This problem is far-reaching because the majority of antibodies presently available recognize a target located on both normal and diseased cells (Slavin-Chiorini, et al., 5 Int. J. Cancer 53: 97-103 (1993)).

The constant region can promote cell death through antibody dependent cell mediated cytotoxicity (ADCC) or by complement dependent cytotoxicity (CDC). Despite the deletion of portions of the constant region, particularly the CH<sub>2</sub> domain, 10 the antigen binding function can be retained (D. Yelton, M. Scharf, Mutant monoclonal antibody with alterations in biological functions, J. Exp. Methods 156:1131-1148 (1982)).

Others have generated a CH<sub>2</sub>-deleted antibody (Mueller et al., Proc. Natl. Acad. Sci. 15 USA 87: 5702-5705 (1990)). Their findings provide that the CH<sub>2</sub>-deleted antibody was cleared from the blood of tumor-bearing mice much faster than the corresponding intact antibody. Other in vivo findings also confirmed that a CH<sub>2</sub>- deleted antibody, designated ch14.18DCH2, is a potentially useful reagent for radioimmunodetection of human tumors because of its reduced immunogenicity, 20 increased target specificity, and rapid clearance from circulation (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990)).

Generally, whole antibody molecules are composed of two heavy (H) and two light (L) chains which are held together by covalent bonds (disulfide) and non-covalent 25 interactions. Each chain contains a variable region (V) and a constant region (C). The variable regions at the amino termini of the two chains form the antigen binding region. The constant region of the H chain has three components or domains. Occasionally, the first constant region domain (CH<sub>1</sub>) interacts with the C region of the L chain through hydrophobic interactions and generally a disulfide bond,

depending on isotype. The next C region stretch is the hinge-acting disulfide bond stably introduced between two H chains. The second constant region domain (CH<sub>2</sub>) is adjacent to the hinge region. CH<sub>2</sub> contains sequences important for effector functions of the antibody, such as the sequences responsible for complement fixation, and Fc receptor binding. The third constant region domain (CH<sub>3</sub>) is located at the carboxyl terminus of the H chain, and is considered to play an important role in H chain assembly as well as some C region functions.

Today many antibodies in clinical trials are directed against tumor associated antigens. Most tumor associated antigens are not tumor specific but are also generally found on the cell surface of some normal, non-tumorigenic cells. The clinical use of some antibodies directed against tumor associated antigens are limited because of the toxicity associated with their use. Therefore, there is a need for methods for inhibiting toxicity associated with immunoglobulin use in the field of disease therapy (e.g., therapy for tumors, kidney disease, and the like) and in vivo diagnosis.

We addressed this need by discovering methods for inhibiting or reducing toxicity to normal cells generally associated with immunoglobulin immunotherapy or in vivo diagnosis, wherein the immunoglobulin recognizes both diseased and normal cells. Our discovery involves generating immunoglobulin molecules or Ig fusion proteins having structurally altered constant regions which inhibit or reduce immunoglobulin-induced toxicity.

## 25 SUMMARY OF THE INVENTION

The present invention provides methods for inhibiting immunoglobulin-induced toxicity by using known immunoglobulin or Ig fusion protein molecules which are structurally altered in their constant regions so that the resulting structurally altered

immunoglobulin or Ig fusion protein molecules exhibit reduced or inhibited toxicity in vivo compared to their original unmodified counterparts.

Structural alteration of the constant region may be effected in a number of ways as  
5 long as it results in reducing or inhibiting immunoglobulin-induced toxicity.

In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH<sub>2</sub> domain is deleted. In another embodiment, only that portion of the  
10 CH<sub>2</sub> domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH<sub>2</sub> domain that binds the complement component C1q is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.

15 Alternatively, structural alteration is effected by single or multiple mutations in the CH<sub>2</sub> domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC  
20 response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

Further alternatively, structural alteration can be effected by isotype switching  
25 resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 is a line graph showing plasma clearance in high  $Le^Y$  expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

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Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the 10 human (h)BR96-light chain (SEQ ID NO. 13).

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the CH<sub>2</sub> deletion (PCT Application No. 95/305444, published March 6, 1996)).

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Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH<sub>2</sub> deletion (PCT Application No. 95/305444, published March 6, 1996)).

20 Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96.

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to  $Le^Y$  (closed diamond), (2) hBR96-2A to  $Le^Y$  (96:0006A2 R/A)(closed square), (3) hBR96-2A to  $Le^Y$  (96:0006B R/A)(closed triangle), and BR96-Dox to 25  $Le^Y$  (X).

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to  $Le^Y$  (closed diamond), (2) chiBR96 to  $Le^Y$  (closed square), (3) cBR96-A to  $Le^Y$  (96:0003 R/A)(closed triangle), and cBR96-Dox to  $Le^Y$  (X).

Figures 9a-c are schematic diagrams showing the steps for deleting a CH<sub>2</sub> domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH<sub>2</sub>  
5 domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.

10 Figure 13 is a schematic diagram showing the construction of pD17-hJm14-  
dCH2.H1.

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in  
15 Figure 5, chimeric BR96 having the CH<sub>2</sub> deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole  
chiBR96 and deleted CH<sub>2</sub> chiBR96 on Le<sup>y</sup>.

20 Figure 16 is a description of the seven structural alterations.

Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.

25 Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the  
legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b mAb, negative control.

5 Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b monoclonal antibody (mAb), negative control.

10

Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH<sub>2</sub> deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

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Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X 20 trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH<sub>2</sub> deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

25

Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of the 1.4 kpb IgG heavy chain region showing the hinge CH<sub>2</sub> and CH<sub>3</sub> domains as boxed regions. Site-specific mutations to be introduced into CH<sub>2</sub> positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (\*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' 5 ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR produces as is shown in the four-way recombination of RsA2, A1B1, B1Ra with 10 vector.

Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve 15 randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable 20 region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

25 Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH<sub>2</sub> domain.

**DETAILED DESCRIPTION OF THE INVENTION****DEFINITIONS**

5 As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at 10 elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

15 The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by 20 symptoms other than those described above.

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and 25 monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant 5 genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity 10 associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated 15 domain.

Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of 20 structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural 25 alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH<sub>2</sub> domain of the constant region. In this instance, deletion of the entire CH<sub>2</sub> domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the

molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of  
5 the CH<sub>2</sub> domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH<sub>2</sub> domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity.

10 For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

Alternatively, structural alteration can be effected by isotype switching (also known  
15 as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-  
20 human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) *Annu. Rev. Immunol.* 8:303-333; T. Honjo et al. (1979) *Cell* 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

25 As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, 5 tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 10 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

As used herein, "treating" means to (1) provide tumor regression so that the tumor is 15 not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, 20 immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or 25 subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including 5 coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may 10 also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone 15 PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

## 20 METHODS OF THE PRESENT INVENTION

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize 25 the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

5

In one embodiment of the invention, the immunoglobulin molecule recognizes and binds  $Le^y$ . In another embodiment, the immunoglobulin recognizes and binds  $Le^x$ .

In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type

10 Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10036. In yet another embodiment, the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10460.

15

In accordance with the practice of the invention, the immunoglobulin can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the

20 ATCC binds. Also, in accordance with the practice of the invention, the immunoglobulin can be an anti-idiotypic antibody.

As required by the invention, at least a portion of the constant region of the immunoglobulin molecule is structurally altered. Structural alteration can be 25 effected by a number of means. In one embodiment, the entire constant region, i.e.,  $CH_1$ ,  $CH_2$ , and  $CH_3$  domains, can be deleted.

In another embodiment, only the  $CH_2$  domain is deleted from the immunoglobulin molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4). In this embodiment, the

CH<sub>2</sub> deletion may result in a molecule unable to bind the Fc receptor or a complement component.

In another embodiment, only that portion of the CH<sub>2</sub> domain which binds the 5 complement component C1q is deleted. In yet another embodiment, mutations in specific portions of the CH<sub>2</sub> domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited. A discussion of such mutations are further found hereinafter.

10

Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a 15 CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a <sup>51</sup>Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC 20 response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

In another embodiment of the invention, the method comprises administering to the 25 subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH<sub>2</sub> domain so that the altered molecule no longer binds the Fc receptor or a complement component.

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one 5 embodiment, the antibody recognizes and binds Le<sup>y</sup>. In another embodiment, the antibody recognizes and binds to Le<sup>x</sup>.

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of 10 HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma 15 having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a 20 subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

25 This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated 5 domains in the CH<sub>2</sub> domain of the constant region of the Ig protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates 10 symptoms associated with the disease.

In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH<sub>2</sub> domain of the constant region of 15 the Ig protein by the same means discussed above.

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as 20 chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known 25 (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

5

Alternatively, the structurally altered antibody or Ig fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as  $^{131}\text{I}$ ; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, "Analysis, Results, And Future Prospective Of The Therapeutic Use Of

10 Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)). According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

15

Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in

20 combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates", 25 Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH<sub>2</sub> domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical 5 compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein 10 or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of 15 administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include, 20 but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

25 The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent 5 is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for 10 example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

15 Additionally, in accordance with the invention, the lipid carrier can be a liposome. As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

20 The most effective mode of administration and dosage regimen for the compositions of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

25 The interrelationship of dosages for animals of various sizes and species and humans based on mg/m<sup>2</sup> of surface area is described by Freireich, E.J., et al. Cancer Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

administered daily or proportionally reduced depending on the specific therapeutic situation).

## THE MOLECULES OF THE INVENTION

5

The present invention provides structurally altered BR96 or BR96 Ig fusion proteins. Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit 10 immunoglobulin-induced toxicity.

Various embodiments of structurally altered BR96 or BR96 Ig fusion proteins have been made.

- 15 In one embodiment, designated cBR96-A, the entire CH<sub>2</sub> domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.
- 20 In another embodiment, designated hBR96-2A, the entire CH<sub>2</sub> domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.
- 25 In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine 5 using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin 10 G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).

15 In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end 20 of the CH<sub>2</sub> domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; and the proline residue located at position 331 is mutated to alanine. 25

In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

In another embodiment, designated hBR96-2H, the leucine residue located at 5 position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

10

Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of 15 structurally altered BR96 which is made.

Several options exists for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the 20 desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such 25 derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

5 Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid 10 (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional 15 structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) 20 are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

25 In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

**NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION**

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region 5 is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons 10 GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

15 In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC, 20 UCA, or UGG.

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU, GCC, GCA, or GCG.

25 In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA 5 (cDNA), or ribonucleic acid (RNA).

## IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or Ig fusion proteins) may be 10 constructed using a wide variety of chemotherapeutic agents such as folic acid and anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of 15 Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)" 85:1189-1193 (1988)), Daunomycin (Arnon and Sela "In Vitro and in vivo Efficacy 20 of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

25 BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).

In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

5

Suitable therapeutic agents for use in making the immunoconjugate includes Pseudomonas exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic 10 agent.

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

15

Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil, decarbazine.

20

Alkylating agents include mechlorethamine, thiotepa, chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin.

25

Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

5 Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons.

Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, suporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium

10 bromide, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapeutic agent

15 aminopterin has a correlative improved analog namely methotrexate.

Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is

20 cyclophosphamide.

## METHODS FOR MAKING MOLECULES OF THE INVENTION

There are multiple approaches to making site specific mutations in the CH<sub>2</sub> domain

25 of an immunoglobulin molecule. One approach entails PCR amplification of the CH<sub>2</sub> domain with the mutations followed by homologous recombination of the mutated CH<sub>2</sub> into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the f1 origin of replication. This gives the vector the properties of a phagemid and site-directed mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

10

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

15

#### EXAMPLE 1

The following standard ELISA protocol was used.

20 **Materials:** Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')<sub>2</sub> 25 Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research), Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le<sup>y</sup>-HSA (Alberta Research Council).

**Methods:** Dilute primary antibody or antigen to 1.0  $\mu$ g/ml in 0.05M Carb/Bicarb buffer. Add 100 $\mu$ l of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

5 Block plates by flicking them and blotting on paper towels. Add 200 $\mu$ l/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

10

Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100 $\mu$ l/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

15

For conjugation add 100  $\mu$ l/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

20

Add 100  $\mu$ l/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N  $H_2SO_4$  100  $\mu$ l/well. Read plate at 450/630nm in EIA plate reader.

## EXAMPLE 2

25

Construction of  $CH_2$  deleted BR96 molecules

**Strategy for Deleting  $CH_2$  Domains:** To construct  $CH_2$  deleted BR96 molecules, the hinge,  $CH_2$  and  $CH_3$  domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH<sub>3</sub> domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pN $\gamma$ 1.14) molecule lacking the CH<sub>2</sub> domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of 5 IgG1 constant region at both sides preserving E.co47-III sites were synthesized. The amplified hinge and CH<sub>3</sub> domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

10

A sewing PCR strategy was used for the construction of CH<sub>2</sub> deleted human IgG1 (pN $\gamma$ 1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH<sub>1</sub> domain was amplified as a 580 bp fragment with a sense oligonucleotide 15 (5' TGG CAC CGA **AAG CTT** TCT GGG GCA GGC CAG GCC TGA 3') (primer A) and an antisense oligonucleotide (5' **TCC GAG CAT GTT GGT ACC CAC GTG GTG GTC GAC** GCT GAG CCT GGC TTC GAG CAG ACA 3') (primer B) from a linearized human IgG1 constant region vector (pN $\gamma$ 1.7). The PCR fragment extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra- 20 III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH<sub>1</sub> domain.

The CH<sub>3</sub> domain was then partially amplified (to the Xba-I site) with a sense primer 25 (5' **GTC GAC CAC CAC GTG GGT ACC AAC ATG TCC GGA GCC ACA TGG ACA GAG GCC GGC T** 3') (primer C) and an antisense primer (5' CTG GTT CTT GTT CAT CTC CTC **TCT AGA TGG** 3') (primer D) from a linearized human IgG1 constant region vector (pN $\gamma$ 1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-1 site (in bold) within the CH<sub>3</sub> domain.

The CH<sub>1</sub> and CH<sub>3</sub> partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and re-annealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH<sub>1</sub> - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH<sub>3</sub> partial - Xba-1.

10

The combined PCR fragment, with the CH<sub>1</sub> and partial CH<sub>3</sub> domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

15

To transfer the CH<sub>1</sub> and partial CH<sub>3</sub> into a mammalian expression vector, both the pEMBL18 and pN $\gamma$ 1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pN $\gamma$ 1.7 vector. The new construct, with CH<sub>1</sub> and a full CH<sub>3</sub> domain, was designated the pN $\gamma$ 1.10 vector.

20

The hinge fragment was amplified from a Hind-III digested pN $\gamma$ 1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH<sub>1</sub> and CH<sub>3</sub> domains of the pN $\gamma$ 1.10 construct. The sense oligonucleotide (5' ACC ATG **GTC GAC** CTC AGA CCT GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT **CAC GTG GTG** TGT CCC TGG ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplification of the hinge fragment (250 bp).

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pN $\gamma$ 1.10 with the CH<sub>2</sub> and CH<sub>3</sub> domains were digested with Sal-1 and Dra-III. The digested hinge 5 fragment was cloned into the Sal-1 and Dra-III linearized sites on the pN $\gamma$ 1.10 vector. The new construct, now carrying the CH<sub>1</sub>, hinge and CH<sub>3</sub> domains, was designated pN $\gamma$ 1.11.

To make the final CH<sub>2</sub> deleted human IgG1 construct, both the pN $\gamma$ 1.11 construct 10 and pN $\gamma$ 1.11 vector were digested with BamH1 and HindIII. A fragment containing the CH<sub>1</sub>, hinge and CH<sub>3</sub> domains was cloned into the linearized pN $\gamma$ 1.11 vector. The new constant region IgG1 construct lacks the CH<sub>2</sub> domain and is designated pN $\gamma$ 1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH<sub>2</sub> and CH<sub>3</sub> domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH<sub>1</sub> and hinge and the 3' end is located inside the CH<sub>3</sub> intron of the BR96 IgG1 molecule. The hinge, CH<sub>2</sub> and CH<sub>3</sub> domains (1.368 kb 20 fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

25

The CH<sub>2</sub> deleted BR96 IgG1 was then constructed as follows. The hinge and CH<sub>3</sub> domains were amplified from a CH<sub>2</sub> deleted L6 IgG1 (pN $\gamma$ 1.14) construct with a sense oligonucleotide (5'

CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTGACCTCAG

A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide  
(5'GGAAAGAACCATCACAGTCTCGCAGGGG  
CCCAGGGCAGCGCTGGGTGCTT 3') homologous to the constant region  
5 sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the p $\gamma$ 1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH<sub>3</sub> domains.

10 The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH<sub>2</sub> and CH<sub>3</sub> domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH<sub>3</sub> PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This  
15 construct lacks the CH<sub>2</sub> domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).

1.9 grams of CH<sub>2</sub>-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

20

### EXAMPLE 3

Toxicity, localization and clearance of CH<sub>2</sub>-deleted chimeric BR96 was tested in vivo as follows.

25

Three dogs received 400 mg/m<sup>2</sup> of cBR96-A, the CH<sub>2</sub> deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of

the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of 5 toxicity.

**Results:** A significant amount of localization of the CH<sub>2</sub> deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m<sup>2</sup>, although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent 10 amounts of intact and CH<sub>2</sub> deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
Localization			
	#271	155	
cBR96			135
	#272	114	
	#273	126	
cBR96-A			89
	#274	52	

15

Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical 20 signs of toxicity seen at doses of 10 mg/m<sup>2</sup>), even if this difference is real, it could

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran 5 historical frozen tissues from dogs treated with native cBR96 or F(ab)2/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A, 10 these data indicate that the CH<sub>2</sub> domain is associated with the induction of acute gastroenteropathy, and that the removal of this domain prevents the induction of gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')2 is not toxic in the dog model 15 and that the toxicity is mediated by the constant region. The CH<sub>2</sub> deletion mutant is a candidate for targeting agents clinically. Because of the very long half-life of chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le<sup>Y</sup> 20 expressing dogs. The study used chimeric versus constant region mutant of cBR96-2.

CBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid 25 clearance. More than enough of the cBR96-A localized to have caused toxicity.

**Discussion:** The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

In man the bleeding is limited to the fundus of the stomach, causing erosion of the 5 superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity.

This toxicity is mediated in man and dog by the antibody molecule alone. At higher 10 doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')2 molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

15

The CH<sub>2</sub> domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH<sub>2</sub> domain would result in immunoglobulin-induced toxicity inhibition.

20 Toxicology study of hBR96-2B

The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m<sup>2</sup> did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had 25 normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

**EXAMPLE 4**

The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The

5 rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M.

10 Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. *Bio/Technology* 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96

15 *Fab. J. Biol. Chem.* 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, Fc $\gamma$ RI and Fc $\gamma$ RIII binding. *Immunology*. 86:319-324).

20 As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH<sub>2</sub> constant domain of human IgG<sub>1</sub>. Six specific amino acid residues distributed throughout the CH2 domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-

25 terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, Fc $\gamma$ RI and Fc $\gamma$ RIII binding. *Immunology*. 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for Clq on IgG. *Nature* 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. *J.Exp.Med.* 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six 5 residues. We were interested in constructing a panel of mutant CH<sub>2</sub> domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various *in vitro* methods have been described where PCR is used to simultaneously 10 introduce distally located site-specific mutations within a gene sequence (Ho, S.N., H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. *Gene* 77:51-59; Ge, L. and P. Rudolpf. 1996. Simultaneous introduction of multiple mutations using overlap extention PCR. *BioTechniques* 22:28-30). Alternatively, an *in vivo* procedure termed recombination 15 PCR (RPCR) has also successfully been used for rapidly and efficiently generating distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), *Methods in Molecular Biology*, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for 20 recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. *BioTechniques* 10:62-66). RPCR uses *E. Coli*'s recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. *In vivo* recombination is mediated through the joining of nucleotide sequences designed into 25 the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH<sub>2</sub> domain.

Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. 5 Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. *J. Biol. Chem.* 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by *in vivo* recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by 10 placing homologous ends on DNA using polymerase chain reaction. *BioTechniques* 10:62-66) into vectors pD17-hG1a and pD16-hC $\kappa$ , to form pBR96-hG1a and pBR96-hC $\kappa$  respectively. pD17-hG1a and pD16-hC $\kappa$  are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis 15 to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH<sub>2</sub>-CH<sub>3</sub> domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA).

20 The strategy for introducing multiple mutations within the immunoglobulin CH<sub>2</sub> gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR 25 products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

proportionately. The recombination of neighboring PCR products always occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues.

The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence

5 flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

10 Each L mutation was amplified in a separate PCR reaction. The reaction conditions were 250 ng intact pBR96-hG1a DNA template, 10 ul of 1X *Pfu* buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5

15 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered

20 DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a vector, transfected into Max competent E. coli DH5 $\alpha$  according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

25

The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kbp DNA insert.

5 Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know

10 10 whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

To evaluate the expression of Le $\gamma$  -binding activity of the CH<sub>2</sub> mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6 clones derived from the quadruple recombination reaction exhibiting the predicted diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hC $\kappa$  DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Le $\gamma$  binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok, 20 G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstrom, K.-E. Hellstrom, W.D. Huse and S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. *J.Immunol.* 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le $\gamma$  -reactive IgG. The spectrum of Le $\gamma$  binding activities were all similar to that of native humanized BR96 IgG indicating 25 that the homologously recombined antibodies did not acquire any gross mutations that could affect antigen binding. To confirm that the desired CH<sub>2</sub> mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone

was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a 5 thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCR-generated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The 10 advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several distinct PCR products should permit combinatorial strategies for constructing 15 complexly mutated protein domains as well as broadening the number and location of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs Constructed	PCR Fragments in reaction	HR <sup>a</sup> events	Colonies Analyzed	Cloning Efficiency <sup>b</sup>
2	2	triple	24	45%
2	3	quadruple	24	33%

<sup>a</sup>HR-homologous recombination  
<sup>b</sup>Cloning efficiency (number of clones containing 1.4kbp insert/total number of colonies)

**EXAMPLE 5**

This example provides two methods for introducing site specific mutations into the  
5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant  
region, wherein mutations are introduced using appropriately constructed  
oligonucleotides. The vector receiving the fragment(s) is digested with a restriction  
10 enzyme to linearize the vector. PCR amplification primers are designed so that the  
5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If  
more than one PCR fragment is amplified, then common sequences to the two  
fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR  
fragments and with the digested vector. The fragments and vector can recombine by  
15 homologous recombination using the bacteria's recombination machinery. Bacterial  
colonies are selected and the DNA is analyzed by size and restriction map as a  
preliminary determination that the vector and fragment(s) recombined correctly.  
Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide  
sequence analysis. DNA is then introduced into mammalian cells as described for  
20 the CH2 deleted antibody, and the expressed antibody analyzed for binding and  
functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at  
residue 237 were introduced by the procedure disclosed in Example 4. The heavy  
25 chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector  
described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J.  
Harris, J. Bajorath, K-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin,  
W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three  
affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two Eco47-III restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with *Eco*47-III, (2) isolating the vector by agarose gel electrophoresis, and (3) 5 extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to 10 herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco*47-III digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10  $\mu$ l of 10X *Pfu* 20 buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase (Stratagen, Inc. San Diego, CA) in 100  $\mu$ l reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 25 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final extension at 72°C for 7 min. The amplified products were purified from a 1% agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of each PCR product was mixed with 25 ng of the *Eco*47-III digested pBR96-hG1a vector and transfected in *E.coli* MAX Efficiency DH5 $\alpha$ ™ according to the

manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD).

The entire transfection reaction was plated onto LB agar plated containing 100 µg/ml ampicillin.

- 5 Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).
- 10 The second method for introducing site specific mutations into the CH<sub>2</sub> domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, *supra*). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent *E. coli* CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
- 15 hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.

Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridylated DNA was prepared using the Muta-Gene Phagemid In Vitro

- 20 Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at
- 25 residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared by a combination of these methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3 mutations and the plasmid was used to transfet mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with NheI for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

10

Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

15

The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

**Sens(sense)CH2 E47-3-5:** CAG GGA GGG AGG GTG TCT GCT GGA AGC  
20 CAG GCT CAG CGC TGA CCT CAGA  
**D CH2 E47-3 A (antisense):** GGA AAG AAC CAT CAC AGT CTC GCA GGG  
GCC CAG GGC AGC GCT GGG TGC TT

Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences show sites of mutation):

**Antisense CH2 L235-G237/aa:** GAA GAG GAA GAC TGA CGG TGC CCC  
CGC GAG TTC AGG TGC TGA GG

**SensCH2 L235-G237/AA:** CCT CAG CAC CTG AAC TCG CGG GGG CAC  
CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

**Antis(antisense)CH2 EKK/SSS-2:** CTG GGA GGG CTT TGT TGG AGA CCG  
AGC ACG AGT ACG ACT TGC CAT TCA GCC

5 Oligonucleotides to mutate Pro331 to Ala:

**Antis CH2 P331/A/3:** GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC

**Sense CH2 P33/A:** GCC CTC CCA GGC GCC ATC GAG AAA ACC ATC

Alternative antisense oligo to introduce Ala at 331 by site-directed mutation:

**CH2P331A:** GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

10

Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331

to Ala:

**Antis CH2 EKKP/SSA-6:** GAT GGT TTT CTC GAT GGC GGC TGG GAG  
GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG

15 CCA GTC CTG GTG

**Sense CH2 EKKP/SSA-6:** CAC CAG GAC TGG CTG AAT GGC AAG TCG  
TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC  
GAG AAA ACC ATC

20

In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in

25 H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

5 Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant  
10 region are marked.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION

5

(i) APPLICANT: Bristol-Myers Squibb Co.

10 (ii) TITLE OF THE INVENTION:  
A METHOD FOR INHIBITING  
IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF  
IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

(iii) NUMBER OF SEQUENCES: 13

15

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20 (E) COUNTRY: USA  
(F) ZIP: 90025

(v) COMPUTER READABLE FORM:

25 (A) MEDIUM TYPE: Diskette  
(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: DOS  
(D) SOFTWARE: FastSEQ Version 2.0

30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US97/\_\_\_\_\_  
(B) FILING DATE: 01-AUG-1997  
(C) CLASSIFICATION:

35

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/023,033  
(B) FILING DATE: 02-AUG-1996

40

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45

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50

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

55 (A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:  
5 TGGCACCGAA AGCTTTCTGG GGCAAGGCCAG GCCTGA 36  
(2) INFORMATION FOR SEQ ID NO:2:  
(i) SEQUENCE CHARACTERISTICS:  
10 (A) LENGTH: 57 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
15 (ii) MOLECULE TYPE: cDNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:  
20 TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA 57  
(2) INFORMATION FOR SEQ ID NO:3:  
(i) SEQUENCE CHARACTERISTICS:  
25 (A) LENGTH: 55 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
30 (ii) MOLECULE TYPE: cDNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
35 GTCGACCACC ACGTGGGTAC CAACATGTCC GGAGCCACAT GGACAGAGGC CGGCT 55  
(2) INFORMATION FOR SEQ ID NO:4:  
(i) SEQUENCE CHARACTERISTICS:  
40 (A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
45 (ii) MOLECULE TYPE: cDNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  
50 CTGGTTCTTG TTCATCTCCT CTCTAGATGG 30  
(2) INFORMATION FOR SEQ ID NO:5:  
(i) SEQUENCE CHARACTERISTICS:  
55 (A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: cDNA  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACCATGGTCG ACCTCAGACC TGCCAAGAGC CATATC

36

## (2) INFORMATION FOR SEQ ID NO:6:

5

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10

## (ii) MOLECULE TYPE: cDNA

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATGGTCACG TGGTGTGTCC CTGGATGCAG GCTACTCTA

39

## (2) INFORMATION FOR SEQ ID NO:7:

20

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

30

CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA

49

## (2) INFORMATION FOR SEQ ID NO:8:

35

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

45

GGAAAGAACCC ATCACAGTCT CGCAGGGCC CAGGGCAGCG CTGGGTGCTT

50

## (2) INFORMATION FOR SEQ ID NO:9:

50

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8691 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

55

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGTAA  
CCTTTTTTTT TAATTTTATT TTATTTTATT TTTGAGATGG AGTTTGGCGC CGATCTCCCG

60

120

5	ATCCCCATG GTCGACTCTC AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAGTATC TGCTCCCTGC TTGTGTGTTG GAGGTCGCTG AGTAGTGCAG GAGCAAATT TAAGCTACAA CAAGGCAAGG CTTGACCGAC AATTGCATGA AGAATCTGCT TAGGGTTAGG CGTTTTGCGC TGCTTCGCGA TGTACGGGCC AGATATAACGC GTTGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA CCCCATATAT GGAGTTCGGC GTTACATAAC TTACGGTAA TGGCCCGCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCCATAGTA ACGCCAATAG GGACTTCCA TTGACGTAA TGGGTGGACT ATTTACGGTA AACTGCCAC TTGGCAGTAC ATCAAGTGTAA TCATATGCCA AGTACGCC CTATTGACGT CAATGACGGT AAATGGCCCG CCTGGCATTAA TGCCCAGTAC ATGACCTTAT 10 GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTTATTACG ATGGTGTATGC GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGAG CTCACGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTGTG TTTGGCACCA AAATCAACGG GACTTTCCA AATGTGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTAA CGGTGGGAGG TCTATATAAG CAGAGCTCTC TGGCTAACTA GAGAACCCAC TGCTTACTGG CTTATCGAAA 15 TTAATACGAC TCACTATAGG GAGACCCAAAG CTTGGTACCA ATTAAATTG ATATCTCCTT AGGTCTCGAG TCTCTAGATA ACCGGTCAAT CGATTGGAAAT TCTTGCAGGC GCTTGCTAGC CACCAGGAG TGTGGTTAA GCTTGGTCTC TCTTGTCTC TGTTTTAAA GGTGTCCAGT GTGAAGTGA TCTGGTGGAG TCTGGGGAG GCTTAGTGCAG GCCTGGAGGG TCCCTGAAAG TCTCTGTGT AACCTCTGGA TTCACTTTCA GTGACTTATA CATGTATTGG GTTCCGCAGA 20 CTCCAGAGAA GAGGCTGGAG TGGGTGCGAT ACATTAGTCAG AGGTGGTGTATAACCGACT ATCCAGACAC TGTAAGGGT CGATTCACCA TCTCCAGAGA CAATGCCAAG AACACCCCTGT ACCTGCAAT GAGCCGTCTG AAGTCTGAGG ACACAGCCAT GTATTACTGT GCAAGAGGCC TGGACGACGG GGCCTGGTT GCTTACTGGG GCCAAGGGAC TCTGGTCACG GTCTCTGTAG CTAGCACCAA GGGCCCATCG GTCTTCCCCC TGGCACCCCTC CTCCAAGAGC ACCTCTGGGG 25 GCACAGCCGC CCTGGGTGCA CTGGTCAAGG ACTACTTCCC CGAACCCGTG ACGGTGTCTG GGAACTCAGG CGCCCTGACC AGCCGGTGC ACACCTTCCC GGCTGTCTCA CAGTCCCTCAG GACTCTACTC CCTCAGCAGC GTGGTCACCG TGCCCTCCAG CAGCTTGGGC ACCCAGACCT ACATCTGCAA CGTGAATCAG AAGCCCAGCA ACACAAAGGT GGACAAGAAA GTTGGTGAGA GGCCAGCACA GGGAGGGAGG GTGCTGTG GAAGCAGGGC TGAGCCTCC TGCCTGGACG 30 CATCCCGCT ATGCAGCCCG AGTCCAGGG AGCAAGGGAG GCCCCGTCTG CCTCTTCACC CGGAGGCCCTC TGCCCCCCCCC ACTCATGCTC AGGGAGAGGG TCTTCTGGCT TTTTCCCCAG GCTCTGGCA GGCACAGGT AGGTGCCCCCT AACCCAGGG CTGCACACAA AGGGGCAGGT GCTGGCTCA GACCTGCAAG GAGCCATATC CGGGAGGACCT CTGCCCCCTGA CCTAAGCCCC CCCCAAAGGC CAAACTCTCC ACTCCCTCAG CTGGACACCC TTCTCTCTC CCAGATTCCA 35 GTAATCTCCA ATCTCTCTC TGAGAGGCC AAATCTGTG ACAAAACCTCA CACATGCCCA CCCTGCCAG GTAAAGCCAGC CCAGGCCCTCG CCCTCCAGCT CAAGGCCGG AAGGTGCCCT AGAGTAGCCT GCATCCAGGG ACAGGCCCA GCGGGGTGCT GACACGTCCA CCTCCATCTC TTCTCTAGCA CCTGAACCTCC TGCCCCGACCC GTCAGTCTC CTCTTCCCCC CAAAACCCAA GGACCCCTC ATGATCTCCC GGACCCCTGA GGTCACTGTC GTGGTGGTGG ACGTGAGCCA 40 CGAAGACCCCT GAGGTCAAGT TCAACTGCTA CGTGGACGGC GTGGAGGTGCA ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCCT CTGCACCCAG GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAAGCCCT CCCAAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGT GGGACCCGTG GGGTGCAGG GCCACATGGA CAGAGGCCCG CCTGGCCCTC CCTCTGCCCT CAGAGTGCAC GCTGTACCAA 45 CCTCTGTCCC TACAGGGCAG CCCCGAGAAC CACAGGTGTA CACCCCTGCC CCATCCGGG ATGAGCTGAC CAAGAACCG AGTCAACAG CTCAGCTCTGA CCTGCTGGT CAAAGGCTTC TATCCCAGCG ACATGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCC CCGTGTGGA CTCCGACGGC TCCTCTCTC TCTACAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGTGCA TGAGGCTCTG CACAACCACT 50 ACACGCCAGAA GAGCCCTCTC CTGCTCTCCGG GTAAATGAGT GCGACGCCCG GCAAGCCCC GCTCCCCGGG CTCTCGCGGT CGCACGAGGA TGCTTGGCAC GTACCCCTG TACATACCTC CCGGGGCCCG AGCATGGAAA TAAAGCACCC AGCGCTGCC CGGGCCCTG CGAGACTGTG ATGGTTCTTT CCACGGGTCA GGCGAGGTCT GAGGCCTGAG TGGCATGAGG GAGGAGAGC GGGTCCCACCT GTCCCCACAC TGCCCCAGGC TGTCAGGTG TGCTGGGCC CCCTAGGGTG 55 GGGCTCAGCC AGGGGCTGCC CTGGCAGGG TGGGGGATTT GCCAGCGTGG CCCTCCCTCC AGCAGCACCT GCCCTGGGCT GGGCCACCGG AAGCCCTAGG AGCCCTGGG GACAGACACA CAGCCCCCTGC CTCTGTAGGA GACTGTCTG TTCTGTGAGG GCCCCGTCTC TCCCGACCTC CATGCCCACT CGGGGGCATG CCTAGTCCAT GTGCGTAGGG ACAGGCCCTC CCTCACCC CTACCCCCAC GGCACTAACC CCTGGCTGCC CTGGCAGGCC TCGCACCCGC ATGGGGACAC	180 240 300 360 420 480 540 600 660 720 780 840 900 960 1020 1080 1140 1200 1260 1320 1380 1440 1500 1560 1620 1680 1740 1800 1860 1920 1980 2040 2100 2160 2220 2280 2340 2400 2460 2520 2580 2640 2700 2760 2820 2880 2940 3000 3060 3120 3180 3240 3300 3360 3420 3480 3540 3600 3660
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	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCACAA	3720
	CACACACTCA	GCCCGACACC	GTTCACACAAA	CCCGCGACTG	AGGTTGGCCG	GCCACACGGC	3780
	CACCCACAC	ACACGTGAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	3840
	CCCAGACCG	AGCAAGGTCC	TCGCACACGT	GAACACTCTC	CGGACACAGG	CCCCCACCGAG	3900
5	CCCCACCGG	CACCTCAAGG	CCCGAGGCC	TCTCGGCAGG	TTCTCCACAT	GCTGACCTGC	3960
	TCAGACAAAC	CCAGCCCTCC	TCTCACAAAGG	GTGCCCCCTGC	AGCCGCCACA	CACACACAGG	4020
	GGATCACACA	CCACGTCACG	TCCCCTGGCC	TGGCCCACTT	CCAGTGGCCG	CCCTTCCCTG	4080
	CAGGAGGAT	CAGCCTCGAC	TGTGCCCTCT	AGTGGCAGC	CATCTGTTGT	TTGCCCCCTCC	4140
	CCCGTGCCTT	CCTTGACCCCT	GGAAAGGTGCC	ACTCCCACGT	TCCTTTCTTA	ATAAAATGAG	4200
10	GAAATTGCA	CGCATTGCT	GAGTAGGTGT	CATTCTATT	TGGGGGGTGG	GGTGGGGCAG	4260
	GACAGCAAGG	GGGAGGATTG	GGAAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	4320
	ATGGCTTCTG	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGGCCCTGT	4380
	AGCGGCGCAT	TAAGCGGCC	GGGTGTGGTG	GTACCGCGCA	CGGTGACCCG	TACACTGCC	4440
	AGCGCCCTAG	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCT	TTCTCGCCAC	TTTCGCCGGG	4500
15	CCTCTCAAAA	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAC	AGTCCCAGCC	4560
	CTAACTCCGC	CCATCCCGCC	CCTAACTCCG	CCCAGTCCG	CCCATCTCC	GCCCCATGGC	4620
	TGACTAATT	TTTTTATTTA	TGCAAGAGGCC	GAGGCCGCCT	CGGCCTCTGA	GCTATTCCAG	4680
	AAGTAGTGTG	GAGGCTTTTT	TGGAGGCCCTA	GGCTTTGCA	AAAAGCTTGG	ACAGCTCAGG	4740
	GCTGCGATT	CGCGCCAAAC	TTGACGGCAA	TCTCTAGCGT	AAGGCTGGTA	GGATTTTATC	4800
20	CCCCGTCGCA	TCATGGTTCG	ACCATTGAAC	TGCTAGCGTC	CCGTGCTCCA	AAATATGGG	4860
	ATTGGCAAGA	ACGGAGACCT	ACCCCTGGCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCAA	4920
	AGAATGACCA	CAACCTCTTC	AGTGGAGGT	AAACAGAAC	TGGTATTAT	GGGTAGGAAA	4980
	ACCTGTTCT	CCATTCTGA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	5040
	AGTAGAGAAC	TCAAAGAAC	ACACGAGGA	GCTCATTTTC	TTGCAAAAG	TTGGATGAT	5100
25	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	5160
	GGAGGCAGTT	CTGTTTACCA	GGAAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	5220
	ACAAGGATCA	TGCGGAATT	TGAAAGTGAC	ACGTTTTCC	CAGAAATTGA	TTTGGGAAA	5280
	TATAAACTTC	TCCCAGAATA	CCCAGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	5340
	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTT	CAAGTTCTCT	5400
30	GCTCCCCCTCC	AAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	5460
	TCTTTGTGAA	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAAACTA	CCTACAGAGA	5520
	TTTAAAGCTC	TAAGGTAAT	ATAAAATTTT	TAAGTGTATA	ATGTGTTAAA	CTACTGATT	5580
	TAATGTTTG	TGTATTAG	ATTCCAACCT	ATGGAACGT	TGAATGGGAG	CAGTGGTGG	5640
	ATGCCCTTAA	TGAGGAAAAC	CTGTTTGCT	CGAGAAGAAT	GCCATCTAGT	GATGATGAGG	5700
35	CTACTGCTGA	CTCTCAACAT	TCTACTCC	CAAAAGAAA	GAGAAAGGT	GAAGACCCCA	5760
	AGGACTTTTC	TCAGAATTG	CTAAGTTTT	TGAGTCATG	TGTGTTAGT	AATAGAACTC	5820
	TTGCTTGCTT	TGCTATTAC	ACCAACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	5880
	TGGAAAATA	TTCTGTAAAC	TTTATAAGTA	GGCATACAG	TTATAATCAT	AACATACTGT	5940
	TTTTTCTTAC	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	AAAAAATTGT	6000
40	GTACCTTTAG	CTTTTAATT	TGAAAGGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCT	6060
	TGACTAGAGA	TCATAATCAG	CCATACAC	TTTGAGAGG	TTTTACTTGC	TTTAAAAAAC	6120
	CTCCCCACACC	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	6180
	TTTATTGCA	CTTATAATGG	TTACAATAA	AGCAATAGCA	TCACAAATT	CACAAATAAA	6240
	GCATTTTTT	CACTGCATTC	TAGTTGTG	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	6300
45	GTCTGGATCG	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCAC	6360
	CCCAACTTGT	TTATTGCA	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTC	6420
	ACAAATAAA	CATTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCTAAACT	CATCAATGTA	6480
	TCTTATCATC	TCTGTATACC	GTGCGCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	6540
	CTGTTTCTG	TGTGAATTG	TTATCCGTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	6600
50	ATAAAGTGT	AAAGCTGGGG	TGCTTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	6660
	TCACTGCCG	CTTCCAGTC	GGGAAACCTG	TGCGGCCAGC	TGCTTAATG	AATCGGCCAA	6720
	CGCGCGGGGA	GAGCGGGTTT	CGTATTGGG	CGCTCTTCGG	CTTCTCGCT	CACTGACTCG	6780
	CTGCGCTCGG	TCGTTGGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	6840
	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAGG	CCAGCAAAG	6900
55	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	CGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	6960
	GAGCATACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	7020
	TACCAAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	7080
	ACCGGATACC	TGTCCGCTT	TCTCCCTTCG	GGAAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	7140
	TGTAGGTATC	TCAGTCGGT	GTAGGTGTT	CGCTCCAAGC	TGGGCTGTG	GCACGAACCC	7200

	CCCGTTCA	CCGACCGCTG	CGCCTTATCC	CGTAACATAC	GTCTTGAGTC	CAACCCGTA	7260
	AGACACCGT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	7320
	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCTAACT	ACGGCTACAC	TAGAAGGACA	7380
5	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	7440
	TGATCCGGCA	AACAAACAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTGCAA	GCAGCAGATT	7500
	ACGCGCAGAA	AAAAGGATC	TCAGAAAGAT	CCTTGTATCT	TTTCTACCGG	GTCTGACGCT	7560
	CAGTGGAAAG	AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAA	AAGGATCTTC	7620
10	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	7680
	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	7740
15	TTTCGTTCAT	CCATAGTTGC	CTGACTCCCC	GTCTGTAGA	TAACTACGAT	ACGGGAGGGC	7800
	TTACCATCTG	CCCCCAGTGC	TGCAATGATA	CCCGAGAC	CACGCTCAC	GGCTCCAGAT	7860
	TTATCAGCAA	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	7920
	TCCGCCTCCA	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGTT	7980
	AATAGTTTG	GCACAGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACCG	CTCGTCGTTT	8040
20	GGTATGGCTT	CATTCACTCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	8100
	TTGTGCAAAA	AAGCGGTTAG	CTCCCTCGGT	CCTCCGATCG	TTGTCAAGG	TAAGTTGGCC	8160
	GCAGTGTAT	CACTCATGGT	TATGGCAGCA	CTGCAATAATT	CTCTTACTGT	CATGCCATCC	8220
	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACAAAGT	CATTCTGAGA	ATAGTGTATG	8280
	CGGCGACCGA	GTTGCTCTTG	CCCCCGTCA	ATACGGATA	ATACCGCGC	ACATAGCAGA	8340
25	ACTTTAAAG	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACCTTC	AAGGATCTTA	8400
	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGAC	CCAACGTATC	TTCAGCATCT	8460
	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAATGC	CGCAAAAAAG	8520
	CCAATAAAGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTCA	ATATTATTGA	8580
	AGCATTATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	8640
	AAACAAATAG	GGGTTCCGCG	CACATTCCC	CGAAAAGTGC	CACCTGACGT	C	8691

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 8327 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## 35 (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCCG	GCTTCGAATA	GCCAGAGTAA	60
40	CCTTTTTTTT	TAATTTTATT	TTATTTATT	TTTGAGATGG	AGTTTGGCGC	CGATCTCCCG	120
	ATCCCCCTATG	GTGCACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTTGTTG	GAGGTGCGCTG	AGTAGTGCCG	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCAATGA	AGAATCTGCT	TAGGTTTGTAGG	CGTTTGCAGC	300
	TGCTTCGCGA	TGTACCGGCC	AGATATACGC	GTTGACATTTG	ATTATTGACT	AGTTATTAAT	360
45	AGTAATCAAT	TACGGGGTCA	TTAGTTCAT	GCCCATAATAT	GGAGTCCGC	GTTACATAAC	420
	TTACGGTAAA	TGGCCCGCT	GGCTGACCGC	CCAACGACCC	CCGCCATTG	ACGTCATAAA	480
	TGACGTATGT	TCCCAGTGA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCAAC	TTGGCAGTAC	ATCAAGTGT	TCATATGCCA	AGTACGCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCG	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	660
50	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTAC	ATGGTGTATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCAGGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGAA	CAACTCCGCC	CCATTGACGC	AAATGGCCG	TAGGCGTGT	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
55	TTAATAACGGAC	TCACTATAGG	GAGACCCAAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCTTGTCT	TGTTTTAAAA	GGTGTCCAGT	1140
	GTGAAGTGA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGC	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATT	CATGTATTG	GTTGCCAGA	1260

CTCCAGAGAA	GAGGCTGGAG	TGGGTGCGAT	ACATTAGTCA	AGGTGGGTGAT	ATAACCGACT	1320	
ATCCAGACAC	TGTAAAGGGT	CGATTCACCA	TCTCCAGAGA	CAATGCCAAG	AAACCCCTGT	1380	
ACCTGCAAAT	GAGCCGTCTG	AAAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GAAGAGGCC	1440	
TGGACGACGG	GGCCTGGTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500	
5	CTAGCACCAA	GGGCCATCG	GTCTTCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCCGGTG	ACGGTGTCTGT	1620
	GGAACTCAGG	CGCCCTGACC	AGCGCGTGC	ACACCTTCCC	GGCTGTCTCA	CAGTCCTCAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCACCG	TGGCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
10	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTGGTGAGA	1800
	GGCCAGCACA	GGGAGGGAGG	GTGCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCCTGGACG	1860
	CATCCCGGCT	ATGCAGCCCC	AGTCAGGGC	AGCAAGGCAG	GCCCCGCTCTG	CCTCTTCACC	1920
	CGGAGGCCTC	TGCCCCCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTGCCCC	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
15	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCCCTGA	CCTAACGCCA	2100
	CCCCAAAGGC	CAAACCTCTC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
	GTAACTCCCA	ATCTTCTCTC	TGCAAGAGCCC	AAATCTTGTG	ACAAAACCTA	CACATGCCA	2220
	CCGTGCCCAAG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCAGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACACACCACG	TGGGTACCAA	CATGTCCGGA	GCCACATGGA	2340
20	CAGAGGCCGG	CTCGGCCCC	CCTCTGCCC	GAGAGTGCACC	GCTGTACCAA	CCTCTGTCCC	2400
	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCTGCCC	CCATCCCAGG	ATGAGCTGAC	2460
	CAAGAACCCAG	GTCAGCTGTA	CCTGCGCTGG	CAAAGGCTTC	TATCCCAGCG	ACATGCCGT	2520
	GGAGTGGGAG	AGCAATGGGC	AGCCCGAGAA	CAACTACAA	ACCACGCC	CCGTGCTGGA	2580
	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GTCACCGTG	GACAAGAGCA	GGTGGCAGCA	2640
25	GGGAAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACACT	ACACCGAGAA	2700
	GAGCCTCTCC	CTGTCCTCGG	GTAAATGAGT	GGCACGGCCG	GCAAGCCCC	GTCCCCCGGG	2760
	CTCTCGCGGT	CGCACGAGGA	TGCTGGCAC	GTACCCCC	TACATACTTC	CCGGGGCGCC	2820
	AGCATGGAAA	AAAAGCACCC	AGCGCTGCC	TGGGCCCTG	CGAGACTGTG	ATGGTTCTTT	2880
	CCACGGGTCA	GGCGGAGTCT	GAGGCTGAG	TGGCATGAG	GAGGCAGAGC	GGGTCCCAC	2940
30	GTCCCCACAC	TGGCCCGAGG	TGTGCAGGTG	TGCGCTGGG	CCCTAGGGTG	GGGCTCAGCC	3000
	AGGGCTGCGC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT	3060
	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCCCTGGG	GACAGACACA	CAGCCCCCTGC	3120
	CTCTGTAGGA	GACTGCTCTG	TTCTGTGAGG	GCCCCTGTCC	TCCCGACCTC	CATGCCCACT	3180
	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGG	ACAGGCC	CCTCACCCAT	CTACCCCCAC	3240
35	GGCACTAAC	CTGGCTGCC	CTGCCCCAGG	TGCAACCGC	ATGGGACAC	AACCGACTCC	3300
	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCACACA	CACACACTCA	3360
	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	CACCACACAC	3420
	ACACGTGAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	CCCAGACCCAG	3480
	AGCAAGGTGTC	TCGCACACGT	GAACACTCTC	CGGACACAGG	CCCCCACGAG	CCCCCACCGG	3540
40	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	TCAGACAAAC	3600
	CCAGCCTCC	TCTCACAAGG	GTGCCCC	AGCGCCACA	CACACACAGG	GGATCACACA	3660
	CCACGTCA	TCCCTGGCCC	TGGCCCAC	CCCAGTGC	CCCTCCCTG	CAGGACGGAT	3720
	CAGCTCGAC	TGTGCTTCT	AGTTGCCAGC	CATCTGTTG	TTGCCCTCC	CCCGTGCCTT	3780
	CCTTGACCC	GGAAAGGTG	ACTCCCAC	TCTTTCTCA	ATAAAATGAG	GAAATTGAT	3840
45	CGCATTGTCT	GAGTAGGTG	CATTCTATT	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	3900
	GGGAGGATTG	GGAAAGCAAT	AGCAGGCATG	CTGGGGATG	GGTGGGCTCT	ATGGCTCTG	3960
	AGGCAGGAAAG	AACCAAGCTG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	AGCGGGCGCAT	4020
	TAAGCGCGC	GGGTGTGGTG	GTTACCGC	GGCGTACCCG	TACACTTGC	AGCGCCCTAG	4080
	CGCCCCCTCC	TTTCGCTTTC	TTCCCTCCT	TTCTCGCCAC	GTTCCCGGG	CCTCTCAAAA	4140
50	AAGGGAAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCC	CTAACCTCCG	4200
	CCATCCCGCC	CCTAAC	CCCAGTCCG	CCCATCTCC	GCCCCATGGC	TGACTAATTT	4260
	TTTTTATTTA	TGCAAGAGGCC	GAGGCC	CCGGCTCTGA	GCTATTCCAG	AAGTAGTGAG	4320
	GAGGTTTTT	TGGAGG	GGCTTTGCA	AAAAGCTTG	ACAGCTCAGG	GTCGCGATT	4380
	CGCGCCAAAC	TTGACGGC	TCCTAGCGT	AAGGCTGGT	GGATTTATC	CCCGCTGCCA	4440
55	TCATGGTTCG	ACCATTGAAC	TGCA	CCGTG	AAATATGGG	ATTGGCAAGA	4500
	ACGGAGACCT	ACCC	CCGCTCAGGA	ACGAGTTCAA	GTACTTCAA	AGAATGACCA	4560
	CAACCTCTC	AGTGG	AAACAGAATC	TGGT	GGTAGGAAA	ACCTGGTTCT	4620
	CCATTCTG	GAAGAATCGA	CCTTAAAGG	ACAGAATTAA	TATAGTTCTC	AGTAGAGAAC	4680
	TCAAAGAAC	ACCACGAGGA	GTCAT	TTGCAAAAG	TTTGGATGAT	GCCTTAAGAC	4740
	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	GGAGGCAAGT	4800

CTGTTTACCA	GGAAGCCATG	AATCAACCA	GCCACCTTAG	ACTCTTGTG	ACAAGGATCA	4860	
TGCAGGAATT	TGAAAGTGAC	ACGTTTTCC	CAGAAATTGA	TTTGGGAA	TATAAACTTC	4920	
TCCAGAATA	CCCAGCGTC	CTCTGTGAGG	TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	4980	
5	TTGAAAGCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTT	CAAGTTCTCT	5040	
TAAAGCTATG	CATTTTATA	AGACCATGGG	ACTTTGCTG	GCTTTAGATC	TCTTGTGAA	5100	
GGAAACCTAC	TTCTGTGGT	TGACATAATT	GGACAAACTA	CCTACAGAGA	TTTAAAGCTC	5160	
TAAGGTAAT	ATAAAATTTT	TAAGTGTATA	ATGTGTTAAA	CTACTGATTC	TAATTGTTG	5220	
TGTATTTAG	ATTCCAACCT	ATGGAACGTGA	TGAATGGGAG	CAGTGGTGGA	ATGCCTTAA	5280	
TGAGGAAAAC	CTGTTTGCT	CAGAAGAAAAT	GCCATCTAGT	GATGATGAGG	CTACTGCTGA	5340	
10	CTCTCAACAT	TCTACTCCCTC	CAAAAAGAA	GAGAAAGGTA	GAAGACCCC	AGGACTTTCC	5400
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TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	TGGAAAATA	5520	
TTCTGTAAAC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTTCTTAC	5580	
15	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	GTACCTTTAG	5640
CTTTTTAATT	TGTAAGGGG	TTAATAAGGA	ATATTGATG	TATAGTGCCT	TGACTAGAGA	5700	
TCATAATTCAG	CCATACCA	TTTGTAGAGG	TTTACTTGC	TTTAAACAC	CTCCCACACC	5760	
TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTG	TGTTAACCTG	TTTATTGCGAG	5820	
CTTATAATGG	TTACAAATAAA	AGCAATAGCA	TCACAAATT	CACAAATAAA	GCATTTTTT	5880	
20	CACTGCATTC	TAGTTGTGGT	TTGTCACAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG	5940
GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCAC	CCCAACTTGT	6000	
TTATTGCGAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTC	ACAATAAAAG	6060	
CATTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCACAACT	CATCAATGTA	TCTTATCATG	6120	
TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCTG	6180	
TGTGAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGT	6240	
25	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTGC	TCACTGCCG	6300
CTTCCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCAATTAGT	AATCGCCAA	CGCGCGGGGA	6360	
GAGGCGGTTT	CGCTATTGGG	CGCTCTTCCG	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	6420	
TCGTTCGGCT	CGGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	6480	
AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAGAAAGG	CCAGCAGAAC	GCCAGGAACC	6540	
30	GTAAAAAGGC	CGCGTTGCTG	GGCTTTTCC	ATAGGCTCG	CCCCCTGAC	GAGCATCACA	6600
AAAATCGACG	CTCAAGTCAG	AGGTGGCAGA	ACCCGACAGG	ACTATAAAGA	TACCAAGCGT	6660	
TTCCCCCTGG	AGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	6720	
TGTCCGCTT	TCTCCCTTCG	GGAAAGCTGG	CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	6780	
TCAGTCGGT	GTAGTCGGT	CGCTCCAAGC	TGGGCTGTG	GCACGAACCC	CCCGTTCA	6840	
35	CGGACCGCTG	CGCTTATCC	GGTAACTATC	GTCTTGACTC	CAACCCGTA	AGACACGACT	6900
TATCGCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	6960	
CTACAGAGTT	CTTGAAGTGG	TGGCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTGTTA	7020	
TCTGCGCTCT	GCTGAAGCCA	GTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	7080	
AACAAACAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTCAA	GCAGCAGATT	ACGCGCAGAA	7140	
40	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAACG	7200
AAAATCAGC	TTAAGGGATT	TTGGTCATGA	GATTATCAA	AAGGATCTTC	ACCTAGATCC	7260	
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ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCACG	GATCTGCTA	TTTCTGTTAT	7380	
CCATAGTTGC	CTGACTCCCC	GTCTGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG	7440	
45	GCCCCAGTC	TGCAATGATA	CCCGCAGACC	CACGCTCAC	GGCTCCAGAT	TTATCAGCAA	7500
TAACCGAGC	AGCGCGAAGG	GCCGAGCGA	GAAGTGGTCC	TGCAACTTCA	TCCGCCCTCA	7560	
TCCAGTCTAT	TAATTGTTG	CGGGAAAGCTA	GAGTAAGTAG	TTGCCAGTT	AATAGTTTG	7620	
GCAACGTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTCA	CTCGTCGTTT	GGTATGGCTT	7680	
CATTCACTC	CGGTTCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCATG	TTGTGCAAAA	7740	
50	AAGCGGTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTGGCC	GCAGTGTAT	7800
CACTCATGGT	TATGGCAGCA	CTGCTAAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT	7860	
TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGGGACCGA	7920	
GTTGCTCTG	CCCGCGTCA	ATACGGGATA	ATACCGGCC	ACATAGCAGA	ACTTTAAAG	7980	
TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACCTC	AAGGATCTTA	CCGCTGTTGA	8040	
55	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACTGATC	TTCAGCATCT	TTTACTTTCA	8100
CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGAAAAAAAG	GGAATAAGGG	8160	
CGACACCGAA	ATGTTGAATA	CTCATACTCT	TCCCTTTCA	ATATTATTGA	AGCATTTATC	8220	
AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	8280	
GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	CCBRAAG		8327	

## (2) INFORMATION FOR SEQ ID NO:11:

5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 8897 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

15	GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGCAC CATGAAGTTG CCTGTTAGGC	60
	TGTTGGTGC GATGTTCTGG ATTCTGCTT CCAGCAGTGA TGTGTTGATG ACCCAAATTC	120
	CAGTCTCCCT GCCTGTCAGT CTTGGAGATC AAGCGTCCAT CTCTTGAGA TCTAGTCAGA	180
	TCATTGTACA TAATAATGGC AACACCTATT TAGAATGGTA CCTGCAGAAA CCAGGCCAGT	240
	CTCCACAGCT CCTGATCTAC AAAGTTTCCA ACCGATTTTC TGGGGTCCCA GACAGGTTCA	300
	GCGGCAGTGG ATCAGGGACA GATTTCACAC TCAAGATCAG CAGAGTGGAG GCTGAGGATC	360
20	TGGGAGTTA TTACTGCTT CAAGGTTCACT ATGTTCCATT CACGTTGCGC TCGGGGACAA	420
	AGTTGGAAAT AAAACGTAAG TCTCGAGTCT CTAGATAACC GGTCAATCGA TTGGAATTCT	480
	AAACTCTGAG GGGGTCGGAT GACGTCGCCA TTCTTGCCT AAAGCATTGA GTTACTGCA	540
	AGGTCAAGAA AGCATGCAAA GCCCTCAGAA TGCGTCAGAA GAGCTCCAAC AAAACAATT	600
	AGAACATTAT TAAGGAATAG GGGGAAGCTA GGAAGAAACT CAAAACATCA AGATTITAAA	660
25	TACGCTTCTT GGTCTCTTG CTATAATTAT CTGGGATAAG CATGCTTT TCTGTCGTC	720
	CCTAACATGC CCTTATCCGC AAACAACACA CCCAAGGGCA GAACTTTGTT ACTTAAACAC	780
	CATCCTGTTT GCTTCTTCC TCAGGAACTG TGGCTGCACC ATCTGCTTC ATCTTCCGC	840
	CATCTGATGA GCAGTTGAAA TCTGGAACTG CCTCTGTTGT GTGCTGCTG AATAACTTCT	900
	ATCCCAGAGA GGCCAAAGTA CAGTGGAAAGG TGATAAACGC CCTCCATCG GTTAACCTCCC	960
30	AGGAGAGTGT CACAGAGCAG GAGAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCTGTA	1020
	CGCTGAGCAA AGCAGACTAC GAGAAACACA AAGTCTACCC CTGCGAAGTC ACCCATCAGG	1080
	GCCTGAGCTC GCCCGTCACA AAGAGCTTC ACAGGGGAGA GTGTTAGAGG GAGAAGTGCC	1140
	CCCACCTGCT CCTCAGTTC AGCCTGACCC CCTCCCATCC TTTGGCCTCT GACCCTTTT	1200
	CCACAGGGGA CCTACCCCTA TTGCGGTCTT CCAGCTCATC TTTCACCTCA CCCCCCTCCT	1260
35	CCTCCTTGGC TTAAATTATG CTAATGTTGG AGGAGAATGA ATAAATAAAG TGAATTTTG	1320
	CACCTGTGGT TTCTCTCTT CCTCATTAA TAATTATTAT CTGTTGTTT ACCAAACTACT	1380
	CAATTCTCT TATAAGGGAC TAAATATGTA GTCATCCTAA GGCACGTAAC CATTATAAA	1440
	AATCATCCTT CATTCTATT TACCCATCA TCCCTCTGCAA GACAGTCCTC CCTCAAACCC	1500
	ACAAGCCTTC TGTCCTCACA GTCCCTTGGG CCATGGTAGG AGAGACTTGC TTCTTGT	1560
40	TCCCCCTCCTC AGCAAGCCCT CATACTGCTT TTAAGGGTG ACAGGTCTTA CAGTCATATA	1620
	TCTTTGATT CAATTCCCTG AGAATCAACC AAAGCAAATT TTTCAAAGA AGAAACCTGC	1680
	TATAAAGAGA ATCATTCTT GCAACATGAT ATAAAATAAC AACACAATAA AAGCAATTAA	1740
	ATAAACAAAC AATAGGGAAA TGTTAAGTT CATCATGGTA CTTAGACTTA ATGAAATGTC	1800
	ATGCCATTATT TACATTTTA AACAGGTACT GAGGGACTCC TGTCTGCCAA GGGCCGTATT	1860
45	GAGTACTTC CACAACCTAA TTTAATCCAC ACTATACTGT GAGATTAAGG ACATTCATTA	1920
	AAATGTTGCA AAGGTTCTAT AAAGCTGAGA GACAAATATA TTCTATAACT CAGCAATCCC	1980
	ACTTCTAGAT GACTGAGTGT CCCCACCCAC CAAAAAAACTA TGCAAGAATG TTCAAAGCAG	2040
	CTTTATTAC AAAAGCCAA AATTGGAAAT AGCCGATTG TCCAAATAA GAATGAGTTA	2100
	TTAAACTGTG GTATGTTAT ACATTTAGAAT ACCAAATGAG GAGAATTAAAC AAGCTACAC	2160
50	TATACCTACT CACACAGATG AATCTCATAA AAATAATGTT ACATAAGAGA AACTCAATGC	2220
	AAAAGATATG TTCTGTATGT TTTCATCCAT ATAAAGTTCA AAACCAAGGTA AAAATAAAGT	2280
	TAGAAATTG GATGGAAATT ACTCTTAGCT GGGGGTGGGC GAGTTAGTGC CTGGGAGAAG	2340
	ACAAGAAGGG GCTTCTGGGG TCTTGGTAAT GTTCTGTTCC TCGTGTGGGG TTGTGCAGTT	2400
	ATGATCTGTG CACTGTTCTG TATACACATT ATGTTCAAA ATAACCTCAC ATAAAGAAC	2460
55	TCTTATACCC AGTTAATAGA TAGAAGAGGA ATAAGTAATA GGTCAAGACC AACGCAGCTG	2520
	GTAAGTGGGG GCCTGGGATC AAATAGCTAC CTGCCTAATC CTGCCWCTT GAGCCCTGAA	2580
	TGAGTCTGCC TTCCAGGGCT CAAGGTGCTC AACAAAACAA CAGGCCTGCT ATTTTCTGG	2640
	CATCTGTGCC CTGTTGGCT AGCTAGGAGC ACACATACAT AGAAATTAAA TGAAACAGAC	2700
	CTTCAGCAAG GGGACAGAGG ACAGAATTAA CCTTGCCAG ACACTGGAAA CCCATGTATG	2760

	AACACTCACA	TGTTTGGGAA	GGGGGAAGGG	CACATGTAAA	TGAGGACTCT	TCCTCATTCT	2820
	ATGGGGCACT	CTGGCCCTGC	CCCTCTCAGC	TACTCATCCA	TCCAACACAC	CTTTCTAAGT	2880
	ACCTCTCTCT	GCCTACACTC	TGAAGGGGTT	CAGGAGTAAC	TAACACAGCA	TCCCTTCCCT	2940
	CAAATGACTG	ACAATCCCTT	TGTCTGCTT	TGTTTTCTT	TCCAGTCAGT	ACTGGGAAAG	3000
5	TGGGAAAGGA	CAGTCATGGA	GAAACTACAT	AAGGAAGCAC	CTTGCCTTC	TGCCCTTGA	3060
	GAATGTTGAT	GAGTATCAAA	TCTTCAAC	TTTGGAGGTT	TGACTAGGGG	TGAGACTCAG	3120
	TAATGTCCT	TCCAATGACA	TGAACTTGC	CACTCATCCC	TGGGGCCAA	ATTGAACAAT	3180
	CAAAGGCAGG	CATAATCCAG	TTATGAATT	TTGCCGCCG	TTGCTAGCTT	CACGTGTTGG	3240
10	ATCCAACCG	GGAAAGGGCCC	TATTCTATAG	TGTACACTAA	ATGCTAGAGC	TCGCTGATCA	3300
	GCCTCGACTG	TGCCTCTAG	TTGCCAGCCA	TCTGTTGTTT	GCCCCCTCCC	CGTGCCTTCC	3360
	TTGACCCCTG	AAGGTGCCAC	TCCCACGTG	CTTTCTAAT	AAAATGAGGA	AATTGCATCG	3420
	CATTGTCGA	GTAGGTGTCA	TTCTATTCTG	GGGGTGGGG	TGGGGCAGGA	CAGCAAGGGG	3480
	GAGGATTGGG	AAGACAATAG	CAGGCATGCT	GGGGATGCGG	TGGGCTCTAT	GGCTTCTGAG	3540
15	GCGGAAAGAA	CCAGCTGGGG	CTCTAGGGGG	TATCCCACG	CGGCCCTGTAG	CGGCCGATTA	3600
	AGCGCGCCG	GTGTGGTGGT	TACCGCGCAGC	GTGACCGCTA	CACTTGCAGC	CGCCCTAGCG	3660
	CCCGCTCCCT	TCGCTTCTT	CCCTTCTTT	CTCGCCACGT	TCGCCGGCC	TCTCAAAAAA	3720
	GGGAAAAAAA	GCATGCATCT	CAATTAGTCA	GCAACCATAG	TCCGCCCT	AACTCCGCC	3780
	ATCCCGCCCC	TAACTCCGC	CAGTCCGCC	CATTCTCCG	CCCATGGCTG	ACTAATTTTT	3840
	TTTATTTATG	CAGAGGCCGA	GGCCGCCCTG	GCCTCTGAGC	TATTCCAGAA	GTAGTGAGGA	3900
20	GGCTTTTTG	GAGGCCCTAG	CTTTGCAA	AAGCTTGGAC	AGCTCAGGGC	TGCGATTTCG	3960
	CGCCAAACTT	GACGGCAATC	CTAGCGTGA	GGCTGTAGG	ATTTTATCCC	CGCTGCCATC	4020
	ATGGTTCGAC	CATTGACTG	CATCGTGC	GTGCCCCAA	ATATGGGAT	TGGCAAGAAC	4080
	GGAGACCTAC	CTTGGCCTCC	GCTCAGGAAC	GAGTTCAAGT	ACTTCCAAAG	AATGACCACA	4140
	ACCTCTTCAG	TGGAAGGTAA	ACAGAACTG	GTGATTATGG	GTAGGAAAAC	CTGGTTCTCC	4200
25	ATTCCTGAGA	AGAATCGACC	TTAAAGGAC	AGAATTAATA	TAGTTCTCAG	TAGAGAACTC	4260
	AAAGAACAC	CACGAGGAGC	TCATTTCTT	GCCAAAGTT	TGGATGATGC	CTTAAGACTT	4320
	ATTGAACAC	CGGAATTGGC	AAAGTAAAGTA	GACATGGTT	GGATAGTCG	AGGCAGTTCT	4380
	GTTTACCAAG	AAGCCATGAA	TCAACCCAGGC	CACCTTAGAC	TCTTTGTGAC	AAGGATCATG	4440
	CAGGAATTG	AAAGTGCAC	GTTCCTCCCA	GAAATTGATT	TGGGAAATA	TAAACTTCTC	4500
30	CCAGAATACC	CAGGGCTCCT	CTCTGAGGT	CAGGAGGAA	AAGGCATCAA	GTATAAGTTT	4560
	GAAGTCTAGC	AGAAGAAAGA	CTAACAGGA	GATGCTTCA	AGTTCTCAG	TCCCCCTCTA	4620
	AAGCTATGCA	TTTTTATAAG	ACCATGGAC	TTTGCTGGC	TTTAGATCTC	TTTGTGAAGG	4680
	AACCTTACTT	CTGTGGTGT	ACATAATTG	ACAAACTACC	TACAGAGATT	TAAGACTCTA	4740
	AGGTTAAAT	AAATTTTTA	AGTGTATAAT	GTGTTAAACT	ACTGATTCTA	ATTGTTGTG	4800
35	TATTTAGAT	TCCAACCTAT	GGAAACTGATG	AATGGGACCA	GTGGTGAAT	GCCTTAAATG	4860
	AGGAAACCT	GTTTGCTCA	GAAGAAATGC	CATCTAGTGA	TGATGAGGCT	ACTGCTGACT	4920
	CTCAACATTC	TACTCCCTCA	AAAAAGAAGA	GAAAGGTAGA	AGACCCCAAG	GACTTCCCT	4980
	CAGAATTGCT	AAAGTTTTG	AGTCATGCTG	TGTTTAGTAA	TAGAACTCTT	GCTTGCTTTG	5040
	CTATTTACAC	CACAAAGGAA	AAAGCTGCAC	TGCTATACAA	GAAAATTATG	AAAAATATT	5100
40	CTGTAACCTT	TATAAGTAGG	CATAACAGT	ATAATCATAA	CATACTGT	TTTCTTACTC	5160
	CACACAGGCA	TAGAGTGTCT	GCTATTAATA	ACTATGCTC	AAAATTGTGT	ACCTTTAGCT	5220
	TTTTAATTG	TAAAGGGGTT	AATAAGGAAT	ATTTGATGTA	TAGTGCCTTG	ACTAGAGATC	5280
	ATAATCAGCC	ATACACATT	TGTAGAGGT	TTACTTGCTT	AAAAAAACCT	CCCACACCTC	5340
	CCCCCTGAACC	TGAACACATAA	AAATGAATGC	ATTGTTGTTG	TTAACATTGTT	TATTGAGCT	5400
45	TATAATGGTT	ACAAATAAAAG	CAATAGCATC	ACAAATTCTCA	CAAATAAAGC	ATTTTTTCA	5460
	CTGCATTCTA	GTTGGGTTT	GTCCAAACTC	ATCAATGTAT	CTTATCATGT	CTGATCGGC	5520
	TGGATGATCC	TCCAGCGCGG	GGATCTCATG	CTGGAGTTCT	TCGCCACCC	CAACTTGT	5580
	ATTGCAGCTT	ATAATGGTTA	CAAATAAAGC	AATAGCATCA	CAAATTTCAC	AAATAAAGCA	5640
	TTTTTTTAC	TGCATTCTAG	TTGTGGTTG	TCCAAACTCA	TCAATGTATC	TTATCATGTC	5700
50	TGTATACCGT	CGACCTCTAG	CTAGAGCTG	CGCTAATCAT	GGTCATAGCT	TTTCTCTGTG	5760
	TGAAATGT	ATCCGCTCAC	AATTCCACAC	AACATACGAG	CCGGAAGC	AAAGTGTAAA	5820
	GCCTGGGTG	CCTAATGAGT	GAGCTAACTC	ACATTAATTG	CGTTCGCTC	ACTGCCGCT	5880
	TTCCAGTCGG	GAACACCTGTC	GTGCCAGCTG	CATTAATGAA	TCGGCCAACG	CGGGGGAGA	5940
	GGCGGTTTGC	GTATTGGGCG	CTCTTCCCGT	TCCTCGCTCA	CTGACTCG	CGCCTCGGT	6000
55	GTTCGGCTGC	GGCGAGCGGT	ATCAGCTCAC	TCAAAGGCGG	TAATACGGTT	ATCCACAGAA	6060
	TCAGGGATA	ACGCAGGAAA	GAACATGTGA	GCAAAAGGCC	AGCAAAAGGC	CAGGAACCGT	6120
	AAAAAGGCCG	CGTTGCTGGC	GTTTTCCAT	AGGCTCCGCC	CCCCCTGACGA	GCATCACAAA	6180
	AATCGACGCT	CAAGTCAGAG	GTGGCGAAAC	CCGACAGGAC	TATAAAGATA	CCAGGCCTTT	6240
	CCCCCTGGAA	GCTCCCTCGT	GGCCTCTCCT	GTTCGGACCC	TGCGCTTAC	CGGATACCTG	6300

	TCCGCCTTTC	TCCCTTCGGG	AAGCGTGGCG	CTTCCTCAAT	GTCACCGCTG	TAGGTATCTC	6360
	AGTCGGTGT	AGTCGTTCG	CTCCAAGCTG	GGCTGTGTGC	ACGAACCCCC	CGTCAGCCC	6420
	GACCGCTCGG	CCTTATCCGG	TAACTATCGT	CTTGAGTCCA	ACCCGGTAAG	ACACGACTTA	6480
5	TCGCCACTGG	CAGCAGCCAC	TGGTAACAGG	ATTAGCAGAG	CGAGGTATGT	AGGCGGTGCT	6540
	ACAGAGTTCT	TGAAGTGGTG	GCCTAACTAC	GGCTACACTA	GAAGGACAGT	ATTTGCTATC	6600
	TGCGCTCTGC	TGAAGCCAGT	TACCTTCGGA	AAAAGAGITG	GTAGCTCTTG	ATCCGGCAAA	6660
	CAAACCACCG	CTGGTAGCGG	TGGTTTTTTT	GTTGCAAGC	AGCAGATTAC	GCAGCAGAAAA	6720
	AAAGGATCTC	AAGAAGATCC	TTTGATCTT	TCTACGGGGT	CTGACGCTCA	GTGGAACGAA	6780
10	AACTCACCGT	AAGGGATTT	GGTCATGAGA	TTATCAAAAA	GGATCTTCAC	CTAGATCCCT	6840
	TTAAATTAAA	AATGAAGTTT	TAAATCAATC	TAAGTATAT	ATGAGTAAAC	TTGGTCTGAC	6900
	AGTTACCAAT	GCTTAATCG	TGAGGCACCT	ATCTCAGCGA	TCTGTCATT	TCGTTCATCC	6960
	ATAGTTGCCT	GAECTCCCGT	CGTGTAGATA	ACTACGATAC	GGGAGGGCTT	ACCATCTGGC	7020
	CCCAGTGCTG	CAATGATACC	GCGAGACCCA	CGCTCACCGG	CTCCAGATT	ATCAGCAATA	7080
15	AACAGGCCAG	CCGGAAGGGC	CGAGCGCAGA	AGTGGTCTG	CAACTTTATC	CGCCTCCATC	7140
	CAGTCTATTA	ATTGTTCCCG	GGAAAGCTAGA	GTAAGTAGTT	CGCCAGTTAA	TAGTTTGCAC	7200
	AAACGGTTG	CCATTGCTAC	AGGCATCGT	GTGTCACGCT	CGTCGTTGG	TATGGCTTCA	7260
	TTCAGCTCCG	GTTCACCGA	ATCAAGGCAG	TTTACATGAT	CCCCATGTT	GTGCAAAAAA	7320
	GCGGTTAGCT	CCTTCGGTCC	TCCGATCGTT	GTCAAGAGTA	AGTGGCCGC	AGTGTATCA	7380
	CTCATGGTTA	TGGCAGCACT	GCATAATTCT	CTTACTGTCA	TGCCATCCGT	AAGATGCTTT	7440
20	TCTGTGACTG	GTGAGTACTC	AACCAAGTCA	TTCTGAGAAT	AGTGTATGCG	GCGACCGAGT	7500
	TGCTCTTGCC	CGCGCTCAAT	ACGGGATAAT	ACCGCGCCAC	ATAGCAGAAC	TTTAAAAGTG	7560
	CTCATCATTG	GAAACCGTTC	TTCGGGGGA	AAACTCTAA	GGATCTTAC	GCTGTTGAGA	7620
	TCCAGTTCGA	TGTAACCCAC	TCGTCACCC	AACTGATCTT	CAGCATCTT	TACTTTCAAC	7680
	AGCGTTCTG	GGTGAGCAA	AACAGGAAGG	AAAATGCCG	AAAAAAAGGG	AATAAGGGCG	7740
25	ACACGGAAAT	GTTGAATACT	CATACTCTTC	CTTTTCAAT	ATTATTGAAG	CATTATATCAG	7800
	GGTTATTGTC	TCATGAGCGG	ATACATATTT	GAATGTATTT	AGAAAAATAA	ACAAATAGGG	7860
	GTTCGGCGCA	CATTTCCCC	AAAAGTGCCA	CCTGACGTCG	ACGGATCGGG	AGATCTGCTA	7920
	GCCCCGGTGA	CCTGAGCGC	GCCGGCTTCG	AAATGCCAGA	GTAACCTTTT	TTTTTAATT	7980
	TATTTTATT	TATTTTGAG	ATGGAGTTG	GCGCGATCT	CCCGATCCCC	TATGGTCGAC	8040
30	TCTCAGTACA	ATCTGCTCTG	ATGCCGCATA	GTTAAGCCAG	TATCTGCTCC	CTGCTTGTG	8100
	GTTGGAGGTC	GCTGAGTAGT	GGCGAGCAA	ATTTAAGCT	ACAACAAAGGC	AAGGCTTGAC	8160
	CGACAATTGC	ATGAAGAAC	TGCTTAGGGT	TAGCGTTTT	GCGCTGCTTC	GCGATGTACG	8220
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	GTCATTAGTT	CATAGCCCAT	ATATGGAGTT	CCCGCTTACA	TAACCTACGG	TAAATGGCCC	8340
35	GCCTGGCTGA	CCGCCCCAACG	ACCCCGAAC	ATTGACGTC	ATAATGACCT	ATGTTCCCAT	8400
	AGTAACGCCA	ATAGGGACTT	TCCATTGACG	TCAATGGGTG	GACTATTAC	GGTAAACTGC	8460
	CCACTTGGCA	GTACATCAAG	TGTATCATAT	GCCAAGTACG	CCCCCTATTG	ACGTCAATGA	8520
	CGGTAATGG	CCCGCCTGGC	ATTATGCCA	GTACATGACC	TTATGGACT	TTCCCTACTTG	8580
	GCAGTACATC	TACGTATTAG	TCATCGCTAT	TACCATGGTG	ATGCGTTTT	GGCAGTACAT	8640
40	CAATGGCGT	GGATAGCGGT	TTGACTCACG	GGGATTCCA	AGTCTCCACC	CCATTGACGT	8700
	CAATGGGAGT	TTGTTTGGC	ACCAAAATCA	ACGGGACTTT	CCAAAATGTC	GTAACAACTC	8760
	CGCCCGATTG	ACGCAAATGG	GCGGTAGGCG	TGTACGGTGG	GAGGTCTATA	TAAGCAGAGC	8820
	TCTCTGGCTA	ACTAGAGAAC	CCACTGCTTA	CTGGCTTATC	GAAATTAATA	CGACTCACTA	8880
	TAGGGAGACC	CAAGCTT					8897

45 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8321 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGTACCAATT	TAAATTGATA	TCTCCTTAGG	TCTCGAGTCT	CTAGATAACC	GGTCAATCGA	60
TTGGAATTCT	TGCGGCCGCT	TGCTAGCCAC	CATGGAGTTG	TGGTTAAGCT	TGGTCTTCCT	120

5	TGTCCCTGTT TTAAAAGGTG TCCAGTGTGA AGTGCACACTG GTGGAGTCCTG GGGGAGGCTT AGTGCAGCCT GGAGGGTCCC TGCAGCTTTC CTGTGCTGCA TCTGGATTCC CGTCAGTGA CTATTACATG TATTGGGTTC GCCAGGCTCC AGGCAAGGGA CTGGAGTGGG TCTCATACAT TAGTCAAGAT GGTGATATAA CCGACTATGC AGACTCCGTA AAGGGTCGAT TCACCATCTC CAGAGACAAT GCAAAAGAACAA GCCTGTACACT GCAAATGAAC AGCCTGAGGG ACGAGGACAC AGCCGTGTAT TACTGTGCAA GAGGCCCTGGC GGACGGGGCC TGTTTGCTT ACTGGGGCCA AGGGACTCTG GTCACGGTCT CTTCCGCTAG CACCAAGGGA CCATCGGTCT TCCCCCTGGC ACCCCTCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGC CTGACCAGCG GCGTGCACAC	180 240 300 360 420 480 540 600 660
10	CTTCCCCGCT GTCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TCACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC AAGAAAGTTG GTGAGAGGCC AGCACAGGG GGGAGGGTGT CTGCTGGAAG CCAGGCTCAG CGCTCCTGCG TGACGCATC CGGGCTATGC AGCCCCAGTC CAGGGCAGCA AGGCAGGGCC CGTCTGCCTC TTACCCCGGA GGCCCTCTGCC CGCCCCACTC ATGCTCAGGG AGAGGGTCTT CTGGCTTTTT CCCCAGGCTC TGCCAGGCC CAGGCTAGGT GCCCCCTAAC CAGGCCCTGC ACACAAAGGG GCAGGTGCTG GGCTCAGGCC TGCCAGAGC CATATCCGGG AGGACCCCTGC CCCGTACCTA AGCCCCACCC AAAGGCCAAA CTCTCCACTC CCTCAGCTCG GACACCTCTC CTCCCTCCAG ATTCAAGTAA CTCCCAATCT TCTCTCTGCA GAGCCCAAAT CTTGTGACAA AACTCACACA TGCCCACCGT GCCCAGGTA GCCAGCCCAG GCCTCGCCCT 20 CCAGCTCAAG GCGGGACAGG TGCCCTAGAG TAGCCTGCAT CCAGGGACAC ACCACGTGGG TACCAACATG TCCGGAGCCA CATGGACAGA GGCCGGCTCG GCCCACCTC TGCCCTGAGA GTGACCGCTG TACCAACCTC TGCCCTACAG GGGCAGCCCC GAGAACACCA GGTGTACACC CTGCCCCCAT CCCGGGATGAG GCTGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAAC 25 TACAAGACCA CGCCTCCCGT GCTGGACTCC GACGGCTCTC TCTTCCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG AACGTCTCTC CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA ATGAGTGCAG CGGCCGGCAAA GCCCCCGCTC CCCGGCTCT CGCGGGTCGA CGAGGATGCT TGGCACGTAC CCCCCTGTACA TACTTCCCGG GCGCCCGAGCA TGAAAATAA GCACCCAGCG CTGCCCTGGG 30 CCCCTGCGAG ACTGTGATGG TTCTTTCCAC GGTCAGGGC GAGTCTGAGG CCTGAGTGGC ATGAGGGAGG CAGAGCGGCTT CCCACTGTCC CCACACTGGC CCAGGCTGTG CAGGCTG TGGGCCCCCT AGGGTGGGGC TCAGCCAGGG GCTGCCCTCG GCAGGGTGGG GGATTG GCGTGGCCCT CCCTCCAGCA GCACCTGCCCC TGGGCTGGGC CACGGGAAGC CCTAGGAGCC CCTGGGGACA GACACACAGC CCCTGCCCT GTAGGAGACT GTCTCTTCT GTGAGCG 35 CTGTCCTCCC GACCTCCATG CCCACTCGGG GGCACTGCCTA GTCCATGTGC GTAGGGACAG GCCCTCCCTC ACCCATCTAC CCCCACGGCA CTAACCCCTG GCTGCCCTGC CCAGCCTCGC ACCCGATGG GGACACAACC GACTCCGGGG ACATGCACTC TCGGCCCTG TGGAGGGACT GGTGCAGATG CCCACACACA CACTCAGCCCC AGACCCGTTT AACAAACCCCC GCACTGAGGT TGGCCGGCCA CACGGCCACC ACACACACAC GTGCACGCC CACACACGGA GCCTCACCCG 40 GGCAGACTGC ACAGCACCA GACCAGAGCA AGGTCTCGC ACACGTGAAC ACTCCCTGG CACAGGCCCTC CACGAGCCCC ACCGGGCACTC TCAAGGCCCA CGAGCCTCTC GGCAGCTTCT CCACATGCTG ACCTGCTCAG ACAAAACCCAG CCCTCCCTCTC ACAAGGGTGC CCCTGCAGCC GCCACACACA CACAGGGGATCACACACAC GTCACGTCCC TGGCCCTGGC CCACCTCCCCA GTGCCCCCTT CCCCCTGAGG AGGGATCAGC CTGACTGTG CCTCTCTAGTT GCCAGCCATC 45 TGTGTTTGC CCCTCCCCCG TGCCCTTCTT GACCCCTGGAA GGTGCCACTC CCACGT TTCTCTTCTTAA AATGAGGAAA TTGCTATGCA TTGCTGAGT AGGTGTCATT CTATTCTGGG GGTGGGGGTG GGGCAGGACA GCAAGGGGGG GGATGGGGAA GACAATAGCA GGCA GGATGCGGTG GGCTCTATGG CTCTGAGGC GGAAAGAAC AGCTGGGGCT CTAGGGGTA TCCCCACCGC CCCTGTAGCG GGCATTAAG CGGGCGGGT GTGGTGGTA CGCGCAGCGT 50 GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTCTTCC CTTCTTCT CGCCACGTTG GCCGGGCTC TCAAAAAAGG GAAAAAAAGC ATGCATCTCA ATTAGTCAGC AACCATAGTC CCGCCCCCTAA CTCCGCCCAT CCCGCCCTA ACTCCGCCA GTTCCGCCA TTCTCCGCC CATGGCTGAC TAATTTTTT TATTTATGCA GAGGCCGAGG CGGCCCTCGC CTCTGAGCTA TTCCAGAAGT AGTGAGGAGG CTTTTTGGA GGCTAGGCT TTTG 55 GCTTGGACAG CTCAGGGCTG CGATTTCGCG CCAAACCTGA CGGCAATCTC AGCGTGAAGG CTGGTAGGAT TTATCCCCG CTGCCATCAT GGTCGACCA TTGAACTGCA TCGTCGCCGT GTCCCAAAAT ATGGGGATTG GCAAGAACGG AGACCTACCC TGCCCTCCGC TCAGGAACGA GTTCAAGTAC TTCCAAAGAA TGACCAACAC CTCTTCAGTG GAAGGTAAC AGAATCTGGT GATTATGGGT AGGAAAACCT GGTTCTCCAT TCCTGAGAACAG AATCGACCTT TAAAGGACAG 3600 3660	2820 2880 2940 3000 3060 3120 3180 3240 3300 3360 3420 3480 3540

	AAATAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCCACCA	CGAGGAGCTC	ATTTCTTGC	3720
	CAAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAAACCG	GAATTGGCAA	GTAAAGTAGA	3780
	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	AACCAGGCCA	3840
	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAAATTGAA	AGTGACACGT	TTTCCCCAGA	3900
5	AATTGATTTG	GGGAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCTCT	CTGAGGTCCA	3960
	GGAGGAAAAAA	GGCATCAAGT	ATAAGTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	4020
	TGCTTCAAG	TTCTCTGCTC	CCCTCTAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT	4080
	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	ATAATTGGAC	4140
	AAACTACCTA	CAGAGATTAA	AAAGCTCTAA	GTAATATAA	AATTTTAAAG	TGATAATGT	4200
10	GTAAAATAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTG	CAACCTATGG	AACTGATGAA	4260
	TGGGAGCAGT	GGTGGAAATGC	CTTTAATGAG	GAAAACCTGT	TTTGTCTAGA	AGAAATGCCA	4320
	TCTAGTGTG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCCTCAA	AAAGAAGAGA	4380
	AAGGAGAAG	ACCCCAAGGA	CTTCTCTCA	GAATTGCTAA	GTGTTTTGAG	TCATGCTGTG	4440
	TTTAGTAATA	GAACTCTTGC	TTGCTTGTCT	ATTACACCA	CAAAGGAAAAA	AGCTGCACTG	4500
15	CTATACAAGA	AAATTATGGA	AAAATATTCT	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	4560
	AATCATAACA	TACTGTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	4620
	TATGCTAAA	AAATTGTGAC	CTTCTAGCTTT	TTAATTGTA	AAAGGGTTAA	TAAGGAATAT	4680
	TTGATGTATA	GTGCCCTGAC	TAGAGATCAT	AATCAGCCAT	ACACACATTG	TAGAGGTTTT	4740
	ACTTTCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	TGAATGCAAT	4800
20	TGTTGTTGTT	AACTTGTGTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	4860
	AAATTTCACA	AAATAAAGCAT	TTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	4920
	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGGGCGGGG	ATCTCATGCT	4980
	GGAGTTCTTC	GGCCACCCCCA	ACTTGTGTTAT	TGCACTTAT	AATGTTACA	AATAAAGCAA	5040
	TAGCATCACA	AATTCACAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	GTGTTTGTC	5100
25	CAAACCTCATC	AATGTATCTT	ATCATGTCG	TATACCGTCG	ACCTCTAGCT	AGAGCTTGGC	5160
	GTAATCATGG	TCATAGCTGT	TTCCCTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	5220
	CATAcgAGCC	GGAAAGCATAA	AGTGTAAAGC	CTGGGGTGC	TAATGAGTGA	GCTAACTCAC	5280
	ATTAATTGCG	TTGCCTCAC	TGCCCCCTT	CCACTCGGGA	AAACCTGTCG	GCCAGCTGCA	5340
	TTAATGAATC	GGCCAACCGC	CGGGGAGAGG	CGGTTTGC	ATTGGGCG	CTTCCGCTTC	5400
30	CTCGCTCACT	GACTCGCTGC	GCTCGGTGCG	TCGGCTGCG	CGAGCGGTAT	CAGCTCACTC	5460
	AAAGGCGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	5520
	AAAAGGCCAG	CAAAGGCCA	GGAAACGTTAA	AAAGGCCGCG	TTGCTGGCG	TTTTCCATAG	5580
	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	5640
	GACAGGACTA	AAAGATACCC	AGGCCTTCC	CCCTGGAAAGC	TCCCTCGTGC	GCTCTCCGT	5700
35	TCCGACCTCG	CCGCTTACCG	GATACCTGTC	CGCCCTTCTC	CCTTCGGAA	GCGTGGCGCT	5760
	TTCTCAATGC	TCACGCTGTA	GGTATCTCG	TTCCGGTGT	GTCGTTGCG	CCAAGCTGGG	5820
	CTGTGTGAC	GAACCCCCCG	TTCACTCGGA	CCGCTGCG	TTATCCGGTA	ACTATCGTCT	5880
	TGAGTCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAACAGGAT	5940
	TAGCAGAGCC	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTG	AAGTGGTGGC	CTAACTACGG	6000
40	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	6060
	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	TTTTTTTTGT	6120
	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTAA	GAAGATCCTT	TGATCTTTTC	6180
	TACGGGGTCT	GACGCTCACT	GGAACGAAAA	CTCACGTTAA	GGGATTTGG	TCATGAGATT	6240
	ATCAAAAGG	ATCTTCACCT	AGATCCTTT	AAATTAAAAA	TGAAGTTTA	AATCAATCTA	6300
45	AGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAACATCGT	AGGCACCTAT	6360
	CTCAGCGATC	TGTCTATTC	GTCATCCAT	AGTTGCTGA	CTCCCCGTCG	TGTAGATAAC	6420
	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGTGCA	ATGATACCGC	GAGACCCACG	6480
	CTCACCGGCT	CCAGATTAT	CAGCAAA	CCAGCCAGCC	GGAGGGCG	AGCGCAGAAG	6540
	TGGCTCTGCA	ACTTTATCG	CCTCCATCCA	GTCATTTAAT	TGTTGCCGG	AAGCTAGAGT	6600
50	AAGTAGTTCG	CCAGTTAATA	GTTCGCGAA	CGTTGTTGCC	ATTGCTACAG	GCATGTTGGT	6660
	GTCACGCTCG	TCGTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	6720
	TACATGATCC	CCCATGTTGT	GCAAAAAAAGC	GGTTAGCTCC	TTCCGGTCTC	CGATCGTTGT	6780
	CAGAAGTAAG	TTGGCCGAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	6840
	TACTGTCATG	CCATCGTAA	GATGTTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	6900
55	CTGAGAATAG	TGTATGCGC	GACCGAGTTG	CTCTTGGCCCG	CGCTCAATAC	GGGATAATAC	6960
	CGCGCCACAT	AGCAGAACTT	TAAGAGTGT	CATCATTGGA	AAACGTTCTT	CGGGCGAAA	7020
	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTGATG	TAACCCACTC	GTGACCCAA	7080
	CTGATCTTCA	GCATTTTTA	CTTTCACCAAG	CGTTTCTGGG	TGAGCAAAA	CAGGAAGGCA	7140
	AAATGCCGCA	AAAAAGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	TACTCTCCT	7200

TTTTCAATAT	TATTGAAGCA	TTTATCAGGG	TTATTGTCTC	ATGAGCCGAT	ACATATTGAA	7260	
ATGTATTCTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	7320	
TGACGTCGAC	GGATCGGGAG	ATCTGCTAGG	TGACCTGAGG	CGCGCCGGCT	TCGAATAGCC	7380	
AGAGTAACCT	TTTTTTTAA	TTTATTTTA	TTTTTATTCTT	GAGATGGAGT	TTGGCGCCGA	7440	
5	TCTCCCGATC	CCCTATGGTC	GACTCTAGT	ACAATCTGCT	CTGATGCCG	ATAGTTAACG	7500
	CAGTATCTGC	TCCCTGCTTG	TGTGTTGGAG	GTCGCTGAGT	AGTGCCTGAG	CAAATTTAA	7560
	GCTACAAACAA	GGCAAGGGCTT	GACCGACAAAT	TGCATGAAGA	ATCTGCTTAG	GGTAGGCGT	7620
	TTTGCCTGTC	TCGCGATGT	ACGGGCCAGA	TATACGCGTT	GACATTGATT	ATTGACTAGT	7680
10	TATTAATAGT	AATCAATTAC	GGGGTCATTA	GTTCATAGCC	CATATATGGA	GTTCGGCGTT	7740
	ACATAACTTA	CGGTAAATGG	CCCGCCTGGC	TGACCGCCCA	ACGACCCCCG	CCCATTGACG	7800
	TCAATAATGA	CGTATGTTCC	CATAGTAACG	CCAATAGGGA	CTTTCCATTG	ACGTCATGG	7860
	GTGGACTATT	TACGGTAAAC	TGCCCCTTG	GCAGTACATC	AAGTGTATCA	TATGCCAAGT	7920
15	ACGCCCCCTA	TTGACGTCAA	TGACGGTAA	TGGCCCGCT	GGCATTATGC	CCAGTACATG	7980
	ACCTTATGGG	ACTTTCTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC	TATTACCATG	8040
	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CCTGGATAGC	GGTTTGACTC	ACGGGGATTT	8100
	CCAAGTCTCC	ACCCCATGAA	CGTCAATGGG	AGTTTGTGTT	GGCACCAAAA	TCAACGGGAC	8160
	TTTCCAAAAT	GTCGTAACAA	CTCCGCCCCA	TTGACGCAA	TGGGCGTAG	GCCTGTACGG	8220
	TGGGAGGTCT	ATATAAGCAG	AGCTCTCTGG	CTAAGTAGAG	AACCCACTGC	TTACTGGCTT	8280
20	ATCGAAATTA	ATACGACTCA	CTATAGGGAG	ACCCAAGCTT	G		8321

20 (2) INFORMATION FOR SEQ ID NO:13:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8897 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GACGGATCGG	GAGATCTGCT	AGCCCGGGTG	ACCTGAGGGG	CGCCGGCTTC	GAATAGCCAG	60	
AGTAACCTTT	TTTTTTAATT	TTATTTTATT	TTATTTTGAA	GATGGAGTTT	GGCGCCGATC	120	
35	TCCCGATCCC	CTATGGTCGA	CTCTCAGTAC	AATCTGCTCT	GATGCCGCAT	AGTTAAGCCA	180
	GTATCTGCTC	CCTGCTTGTG	TGTTGGAGGT	CGCTGAGTAG	TGCGCGAGCA	AAATTTAACG	240
	TACAACAAGG	CAAGGCTTGA	CCGACAAATTG	CATGAAGAAAT	CTGCTTAGGG	TTAGGCCTTT	300
	TGCGCTGCTT	CGCGATGTAC	GGGCCAGATA	TACGCGTTGA	CATTGATTAT	TGACTAGTTA	360
40	TTAATAGTAA	TCAATTACGG	GGTCATTAGT	TCATAGCCCA	TATATGGAGT	TCGGCGTTAC	420
	ATAACTTACG	GTAAATGGCC	CGCCTGGCTG	ACCGCCAAAC	GACCCCGGCC	CATTGACGTC	480
	ATAATGACG	TATGTTCCCA	TAGTAACGCC	AATAGGGACT	TTCCATTGAC	GTCAATGGGT	540
	GGACTATTTA	CGGTTAAACTG	CCCACTTGGC	AGTACATCAA	GTGATACATA	TGCCAAGTAC	600
45	GCCCCCTATT	GACGTCAATG	ACGGTAAATG	GCCCCCTGG	CATTATGCC	AGTACATGAC	660
	CTTATGGGAC	TTTCTACTT	GGCAGTACAT	CTACGTATTA	GTCATCGCTA	TTACCATGGT	720
	GATGCGGTTT	TGGCAGTACA	TCAAATGGCG	TGGATAGCGG	TTTGACTCAC	GGGGATTTC	780
	AAGTCTCCAC	CCCATTGACG	TCAAATGGGAG	TTTGTGTTGG	CACCAAAATC	AACGGGACTT	840
50	TCCAAATGTT	CGTAACAACT	CCGCCCCATT	GACGCAAATG	GGCGGTAGGC	GTGTACGGTG	900
	GGAGGTCTAT	ATAAGCAGAG	CTCTCTGGCT	AACTAGAGAA	CCCACTGCTT	ACTGGCTTAT	960
	CGAAATTAAAT	ACGACTCACT	ATAGGGAGAC	CCAAGCTTGG	TACCAATTAA	AATTGATATC	1020
55	TCCTTAGGTC	TCGAGCACCA	TGAAGTTGCC	TGTTAGGCTG	TTGGTGTCTGA	TGTTCTGGAT	1080
	TCCTGCTCC	AGCAGTGTATG	TTGTATGAC	CCAAACCCCA	CTGTCAGTC	CTGTCAGCCT	1140
	TGGACAACTT	CGCTCCATCT	CTTGCAGATC	TAGTCAGATC	ATTGTACATA	ATAATGGCAA	1200
	CACCTATCTG	GAATGGTACC	AGCAGAGAGCC	AGGGCAGTCT	CCACGGCTCC	TGATCTACAA	1260
	AGTTTCCAAAC	CGATTTCTG	GGGTCCCAGA	CAGGTTCA	GGCAGTGGAG	CTGGGACAGA	1320
	TTTCACACTC	AAGATCAGCA	GAGTGGAGGC	TGAGGATGTG	GGAGTTTACT	ACTGCTTCCA	1380
	GGGTTCCACAT	GTTCCATTCA	CGTTCGGCCA	AGGGACAAAG	TTGGAATCA	AACGTAAGTC	1440
	TCGAGTCTCT	AGATAACCGG	TCAATCGATT	CGAATTCTAA	ACTCTGAGGG	GGTCGGATGA	1500
	CGTGGCCATT	CTTTGCCCTAA	AGCATTGAGT	TTACTGCAAG	GTCAGAAAAG	CATGCAAAGC	1560
	CCTCAGAATG	GCTGCAAAGA	GCTCCAACAA	ACAAATTAG	AACTTTATTA	AGGAATAGGG	1620

5	GGAAGCTAGG AAGAAACTCA AAACATCAAG ATTAAATAATA CGCTTCCTGG TCTCCCTGCT 1680
	ATAATTATCT GGGATAAGCA TGCTGTTTC TGCTGTCCTC TAACATGCCA TTATCCGCAA 1740
	ACAACACACC CAAGGGCAGA ACTTTGTTAC TAAACACCA TCCTGTTTGC TTCTTCCTC 1800
	AGGAACATGTG GCTGACCAT CTGTCTTCAT CTTCCCGCCA TCTGATGAGC AGTTGAAATC 1860
10	TGGAACATGCC TCTGTTGTG GCCTGCTGAA TAACCTCTAT CCCAGAGAGG CCAAAGTACA 1920
	GTGGAAGGTG GATAACGCC CTCATCGGG TAACCTCCAG GAGAGTGTCA CAGAGCAGGA 1980
	GAGCAAGGAC AGCACCTACA GCCTCAGCG CACCCCTGACG CTGAGCAAAG CAGACTACGA 2040
	GAAACACAAA GTCTACGCC GCGAAGTCAC CCATCAGGGC CTGAGCTCGC CCGTCACAAA 2100
	GAGCTTCAAC AGGGGAGAGT GTTAGAGGGA GAAGTGCCTCC CACCTGCTCC TCAGTTCCAG 2160
15	CCTGACCCCCC TCCCCATCCCT TGGCCTCTGA CCCTTTTCTC ACAGGGGACCC TACCCCTATT 2220
	CCGGTCTCC AGCTCATCTT TCACCTCACC CCCCTCCTCC TCCTGGCTT TAATTATGCT 2280
	AATGTTGGAG GAGAATGAAT AAATAAAGTG AATCTTGC A CCTGTTGTTT CTCTCTTCC 2340
	TCATTTAATA ATTATTATCT GTTGTGTTAC CAACTACTCA ATTTCTCTTA TAAGGGACTA 2400
	AATATGTAGT CATCCTAAGG CACGTAACCA TTTATAAAAAA TCATCCTTCAT TTCTTATTTA 2460
20	CCCTATCATC CTCTGAAGA CAGTCCTCCC TCAAAACCCAC AAGCCTCTG TCCTCACAGT 2520
	CCCCTGGGCC ATGGTAGGAG AGACTTGCTT CCTTGTGTTTC CCCTCCTCAG CAAGCCCTCA 2580
	TAGTCTTTTA TAAGGGTAC AGGTCTTACA GTCATATATC CTTTGATTCA ATTCCCTGAG 2640
	AATCAACCAA AGCAAATTTT TCAAAAGAAG AACCTGCTA TAAAGAGAAT CATTCTTGC 2700
	AACATGATAT AAAATAACAA CACAATAAA GCAATTTAAAT AACAAACCAA TAGGGAAATG 2760
25	TTTAAGTTCA TCATGGTACT TAGACTTAAT GGAATGTCTAT GCCTTATTTA CATTCTTAA 2820
	CAGGTACTGA GGGACTCTG TCTGCCAAGG GCCGTATTGA GTACTTTCCA CAACCTAATT 2880
	TAATCCACAC TATACTGTGA GATTAAAAAC ATTCTTAAAT ATGTTGAAAG GGTCTATAA 2940
	AGCTGAGAGA CAAATATATT CTATACTCA GCAATCCCAC TTCTAGATGATGATGTC 3000
	CCACCCACCA AAAAATCTATC CAAGAATGTT CAAAGCAGCT TTATTTACAA AAGCCAAAAA 3060
30	TTGGAAATAG CCCGATTGTC CAACAATAGA ATGAGTTATT AAACGTGGT ATGTTTATAC 3120
	ATTAGAATAC CCAATGAGGA GAATTAACAA GCTACAACTA TACCTACTCA CACAGATGAA 3180
	TCTCATTTAA ATAATGTTAC ATAAGAGAAA CTCATGCAA AAGATATGTT CTGATGTTTT 3240
	TCATCCATAT AAAGTTCAAA ACCAGGTTAA AATAAAGTTA GAAATTGGA TGGAAATTAC 3300
	TCTTAGCTGG GGGTGGGGCA GTTAGTGCCTT GGGAGAAGAC AAGAAGGGGC TTCTGGGGTC 3360
35	TTGGTAATGT TCTGTTCTC GTGTGGGGTT GTGCACTT GATCTGTCCTA CTGTTCTGTA 3420
	TACACATTAT GCTTCAAAAT AACTTACAT AAAACACATC TTATACCCAG TTAATAGATA 3480
	GAAGAGGAAT AAGTAATAGG TCAAGACCAA CGCAGCTGGT AAGTGGGGC CTGGGATCAA 3540
	ATAGCTACCT GCCTAACCT GCCTWCTTGA GCCCTGAATG AGTCTGCCTT CCAGGGCTCA 3600
	AGGTGCTCAA CAAAACAACA GGCTGCTAT TTCTGGCA TCTGTCCTT GTTGGCTAG 3660
40	CTAGGAGCAC ACATACATAG AAATTAATG AAACAGACCT TCAGCAAGGG GACAGAGGAC 3720
	AGAATTAACC TTGCCCAGAC ACTGGAAACC CATGTATGAA CACTCACATG TTTGGGAAGG 3780
	GGGAAGGGCA CATGTAATG AGGACTCTTC CTCACTCTAT GGGGCACTCT GGCCCTGCC 3840
	CTCTCAGCTA CTCACTCCATC CAACACACCT TTCTAACATC CTCTCTCTGC CTACACTCTG 3900
	AAGGGTTCAGGTAACTA ACACAGCATC CTTCCCTCA AATGACTGAC AATCCCTTTG 3960
45	TCCTGTTTG TTTTCTTCAGTCAGTAC TGAAAAGTG GGGAGGACA GTCATGGAGA 4020
	AACTACATAA GGAAGCACCT TGCCCTCTG CCTCTTGAGA ATGTTGATGATGATGAA 4080
	TTTCAAACTT TGGAGGTTTG AGTGGGGTG AGACTCAGTA ATGTCCTCTC CAATGACATG 4140
	AACTTGCTCA CTCATCCCTG GGGGCAAAAT TGAACATCA AAGGCAGGGCA TAATCCAGTT 4200
	ATGAAATTCTT GCGGGCGCTT GCTAGCTTCA CGTGTGGAT CCAACCGGGG AAGGGCCCTA 4260
50	TTCTATAGTG TCACCTAAAT GCTAGAGCTC GCTGATCAGC CTGACTGTG CCTTCTAGTT 4320
	GCCAGCCATC TGTTGTTGC CCCTCCCCCG TGCTTCTCTT GACCTGGAA GGTGCCACTC 4380
	CCACTGTCTT TTCTAAATAA AATGAGGAAA TTGCACTGCA TTGTCAGT GAGTGTCTT 4440
	CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGGG GGATTGGGAA GACAATAGCA 4500
	GGCATGCTGG GGATGCGGTG GGCTCTATGG CTCTGAGGC GGAAAGAAC AGCTGGGGCT 4560
55	CTAGGGGGTA TCCCCACCGCG CCCTGTAGCG GCGCATTAA CGCGGGGGT GTGGTGGTTA 4620
	CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCTTTC GCTTTCTTCC 4680
	CTTCCTTCT CGCCACGTTG GCCGGGCTC TCAAAAAAGG GAAAAAAAGC ATGCACTCA 4740
	ATTAGTCAGC AACCATAGTC CGCCCTAA CTCCGCCCCAT CCCGCCCCCTA ACTCCGCCA 4800
	GTTCCGCCCA TTCTCCGCC CATGGCTGAC TAATTTTTT TATTTATGCA GAGGCCGAGG 4860
	CCGCTCTGGC CTCTGAGCTA TTCCAGAAGT AGTGAGGAGG CTTTTTTGGA GGCCTAGGCT 4920
	TTTGCAAAAAG GCTTGGACAG CTCAGGGCTG CGATTTGCGC CCAAACCTGCA CGCAATCCT 4980
	AGCGTGAAGG CTGGTAGGAT TTATCCCCG CTGCCATCAT GGTTCGACCA TTGAACTGCA 5040
	TCGTCGCCGT GTCCAAAAT ATGGGGATTG GCAAGAACGG AGACCTACCC TGGCCTCCGC 5100
	TCAGGAACGA GTTCAAGTAC TTCCAAAGAA TGACCACAAAC CTCTTCAGTG GAAGGTAAC 5160

5	AGAATCTGGT GATTATGGGT AGGAAAACCT GGTTCTCCAT TCCTGAGAAG AATCGACCTT TAAAGGACAG AATTAATATA GTTCTCAGTA GAGAACTCAA AGAACCAACCA CGAGGAGCTC ATTTCTTGC CAAAAGTTG GATGATGCCT TAAGACTTAT TGAACAACCG GAATTGGCAA GTAAAGTAGA CATGGTTGG ATAGTCGGAG GCAGTTCTGT TTACCAAGGAA GCCATGAATC AACCAGGGCC CCTTAGACTC TTGTGACAA GGATCATGCA GGAATTGAA AGTGACACGT TTTCCCAGA AATTGATTTG GGGAAATATA AACTTCTCCC AGAATAACCCA GGCGTCCCTCT CTGAGGTCCA GGAGGAAAAA GGCACTCAAGT ATAAGTTGA AGTCTACGAG AAGAAAGACT AACAGGAAGA TGCTTCAAG TTCTCTGCTC CCCTCCTAAA GCTATGCATT TTATAAGAC CATGGGACTT TTGCTGGCTT TAGATCTCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC 10 ATAATTGGAC AAACATACCTA CAGAGATTAA AAGCTCTAAAG GTAAATATAA AATTTTAAG TGTATAATGT GTTAAACTAC TGATTCTAAT TGTGTTGTGTTA TTTTAGATTCAACCTATGG AACTGATGAA TGGGAGCAGT GGTGAATGC CTTTAATGAG GAAAACCTGT TTTGCTCAGA AGAAATGCCA TCTAGTGATG ATGAGGCTAC TGCTGACTCT CAACATTCTA CTCCCTCCAAA AAAGAAGAGA AAGGTTAGAAG ACCCCAAGGA CTTCTCTCA GAATTGCTAA GTTTTTGAG TCATGCTGTG TTTAGTAATA GAACCTTGC TTGCTTGCCTT ATTACACCA CAAAGGAAAA AGCTGCACTG CTATACAAGA AAATTATGGA AAAATATTCT GTAACCTTTA TAAGTAGGCA TAACAGTTAT AATCATAACA TACTGTTTT TCTTACTCCA CACAGGCATA GAGTGTCTGC TATTAATAAC TATGCTCAAA AATGTTGAC CTTTAGCTTT TTAAATTGTA AAGGGTTAA TAAGGAATAT TTGATGTATA GTGCCCTGAC TAGAGATCAT AATCAGCCAT ACCACATTG 20 TAGAGGTTTT ACTTGTCTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT TGTGTTGTTA AACTGTTTA TTGCACTTAA TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTCACA AATAAAAGCAT TTTTTCACT GCATTCTAGT TGTGGTTGT CCTAAACTCAT CAATGTATCT TATCATGTCT GGATCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT GGAGTTCTTC GCCCACCCCCA ACTTGTTTAT TGCACTTAA TAATGGTTACA 25 AATAAAGCAA TAGCATCACA AATTTCAAA ATAAGCATT TTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG TATACCGTCA ACCTCTAGCT AGAGCTTGGC GTAATCATGG TCATAGCTGT TTCCCTGTG AAATTGTTAT CCCCTCACAA TTCCACACAA CATACTGAGCC GGAAGCATAA AGTGTAAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC ATTAAATTGCG TTGCGCTCAC TGCCCCCTT CCAGTCGGGA AACCTGTCGT 30 GCCAGCTGCA TTAATGAATC GCCAACCGCG CGGGGAGAGG CGGGGGCGT ATTGGGCGCT CTTCGGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCTG TCGGCTGCGG CGAGGGTAT CAGCTCACTC AAAGGGCGTA ATACGGTTAT CCACAGAAAC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAAACGTAA AAAGGCCGCG TTGCTGGCGT TTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT 35 GGGAAACCC GACAGGACTA TAAAGATACC AGGCCTTTC CCCTGGAAGC TCCCTCGTGC GCTCTCTGT TCCGACCTG CGCCTTACCG GATACCTGTC CGCCTTCTC CCTTCGGAA GCCGTGGCGCT TTCTCAATGC TCACGCTGTA GGTATCTCAG TTGCGGTGAG GTGCTTCGCT CCAAGCTGGG CTGTGTCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAAC CGGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG 40 GTAACAGGAT TAGCAGAGCG AGGTATGTAG CGGGTCTAC AGAGTTCTG AAGTGGTGGC CTAACTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGTAT CGCGCAAACCA AACCCACGCT GGTAGCGGTG GTTTTTTGTG TTGCAACCGAG CAGATTACGC CGACAAAAAA AGGATCTCAA GAAGATCCCT TGATCTTTCTC TACGGGTCT GACGCTCAGT GGAAACGAAA CTCACGTTAA GGGATTTGG 45 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTA AATCAATCTA AAGTATATAT GAGTAAACTT GGTCGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGCTTATTTG GTTCATCCAT AGTTGCTGTA CTCCCCGTG TGAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGTGCA ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG 50 AGCCAGAAG TGGTCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAAT TGTGCTCCGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTTCGCGAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACTGCTCG TCGTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGGCAGT TACATGATCC CCCATGTTGT GCAAAAGC GGTTAGCTCC TTGGTCTCTC CGATCGTGT CAGAAGTAAG TTGGCCGCGAG GTTTCATCACT CATGGTTATG GCAGCACTGC 55 ATAATTCTCT TACTGTCTAG CCATCCGTAAG GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG CTCTTGGCC CGCTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT TAAAGTGTCT CATCATTGGA AAACGTTCTT CGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTGAGATC CAGTTGATG TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTA CTTCACCAAG CGTTCTGGG TGAGCAAAAA 8700	5220 5280 5340 5400 5460 5520 5580 5640 5700 5760 5820 5880 5940 6000 6060 6120 6180 6240 6300 6360 6420 6480 6540 6600 6660 6720 6780 6840 6900 6960 7020 7080 7140 7200 7260 7320 7380 7440 7500 7560 7620 7680 7740 7800 7860 7920 7980 8040 8100 8160 8220 8280 8340 8400 8460 8520 8580 8640 8700
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CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	8760
TACTCTTCCT	TTTCAATAT	TATTGAAGCA	TTTATCAGGG	TTATTGTCTC	ATGAGGGAT	8820
ACATATTTGA	ATGTATTAG	AAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	8880
AAGTGCCACC	TGACGTC					8897

What is claimed is:

1. A method for inhibiting immunoglobulin-induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering an immunoglobulin molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited.  
5
2. A method for inhibiting immunoglobulin-induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering a structurally altered antibody to the subject, the structurally altered antibody comprising a variable region and a constant region, multiple toxicity associated domains in the constant region being modified so as to render the constant region unable to mediate an ADCC response or activate complement thereby inhibiting immunoglobulin-induced toxicity resulting from immunotherapy.  
15
3. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein having multiple structurally altered toxicity associated domains in the constant region.  
20
4. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein comprising a modified constant region, the  
25

modification being a structural alteration in multiple toxicity associated regions within the CH<sub>2</sub> domain.

5. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:

- (a) selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
  
- 10 (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
  
- 15 (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.

20. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:

- 25 (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
  
- (b) structurally altering multiple toxicity associated domains in the CH<sub>2</sub> domain of the constant region of the Ig protein so selected;

5

(c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH<sub>2</sub> domain thereby preventing immunoglobulin-induced toxicity in the subject.

10 7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH<sub>2</sub> domain.

15 8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.

9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.

10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.

11. The method of claim 2, wherein the antibody recognizes and binds Le<sup>y</sup>.

20 12. The method of claim 2, wherein the antibody recognizes and binds to Le<sup>x</sup>.

13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.

25 14. The method of claim 2, wherein the antibody is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds  $Le^y$ .
16. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds to  $Le^x$ .  
5
17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.  
10
18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.  
15
19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds  $Le^y$ .  
20
20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to  $Le^x$ .  
20
21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.  
25
22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.  
23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH<sub>2</sub> domain.

5 24. A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH<sub>2</sub> domain.

10 25. A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.

15 26. The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.

20 27. The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.

25 28. The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.

29. The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the Ig fusion protein is conjugated to a cytotoxic agent.
31. The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected from the group consisting of antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.
32. A method for treating a subject suffering from a cancer, the cancer being characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound thereby curing the subject.
33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH<sub>2</sub> domain.
34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

5

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:

10

(a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and

15

(b) administering the structurally altered BR96 polypeptide of step (a) to the subject under conditions so that the peptide recognizes and binds cancer associated  $Le^y$  antigens, thereby alleviating symptoms associated with the cancer, the structural alteration of the toxicity associated domains thereby preventing BR96 toxicity in the subject.

20

25 37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.
39. A BR96 antibody having humanized variable and constant regions, wherein the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.  
5
40. The BR96 antibody of claim 39 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 12.  
10
41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.  
15
42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.  
20
43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.
- 25 44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid

position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

45. A BR96 antibody designated hBR96-2F having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; and proline at amino acid position 331 is mutated to alanine.
46. A BR96 antibody designated hBR96-2G having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
- 15 47. A BR96 antibody designated hBR96-2H having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
- 20 48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39, and 41-47.
- 25 49. A cDNA of claim 48.
50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.
52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein so produced.

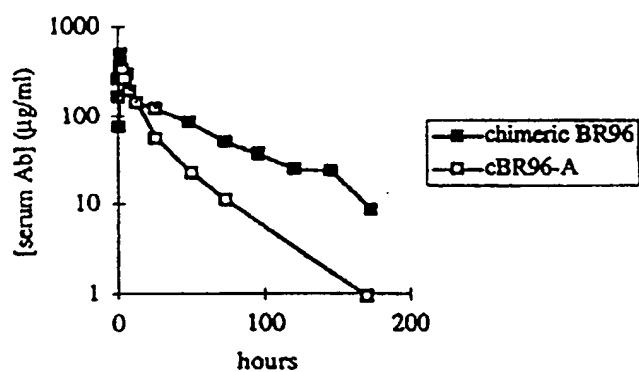
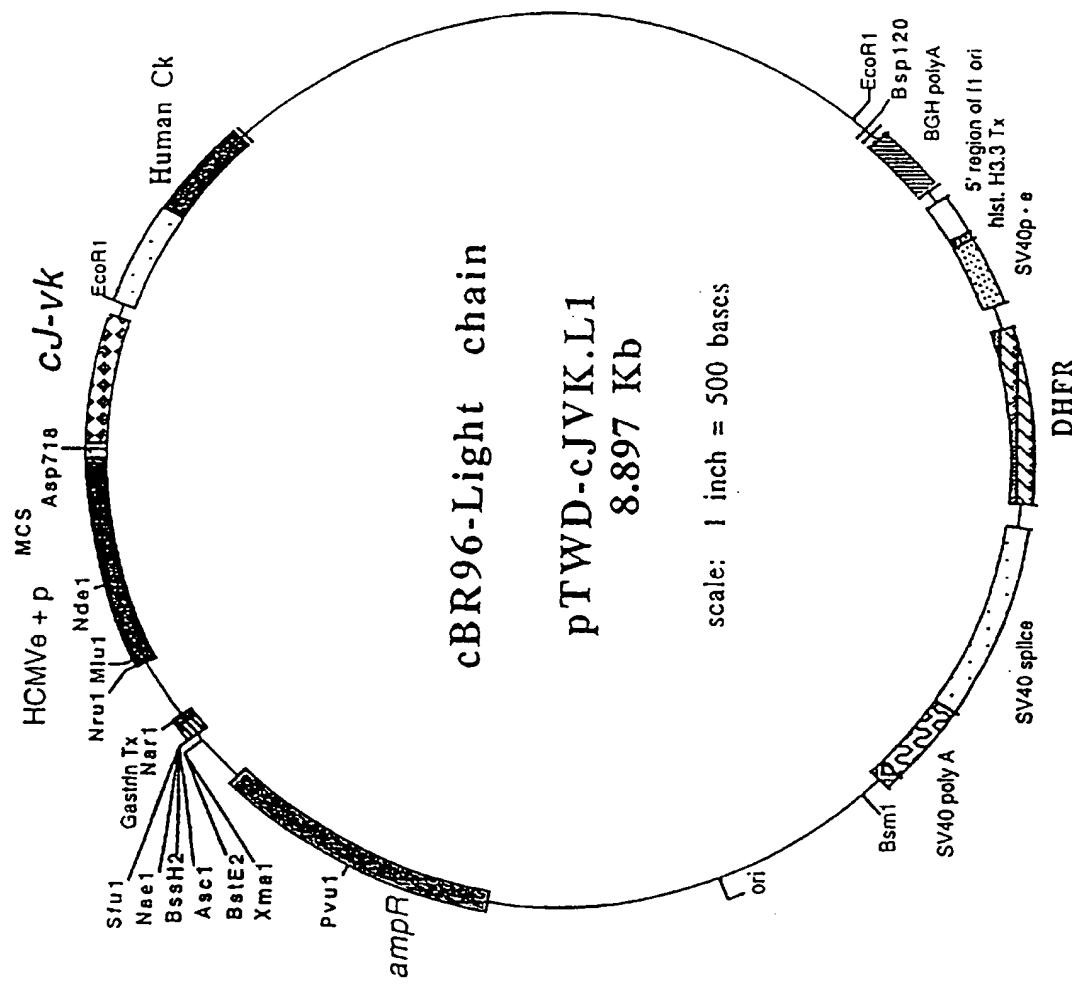


Figure 1. Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

Figure One

1156

Figure 2



2450

Figure 3

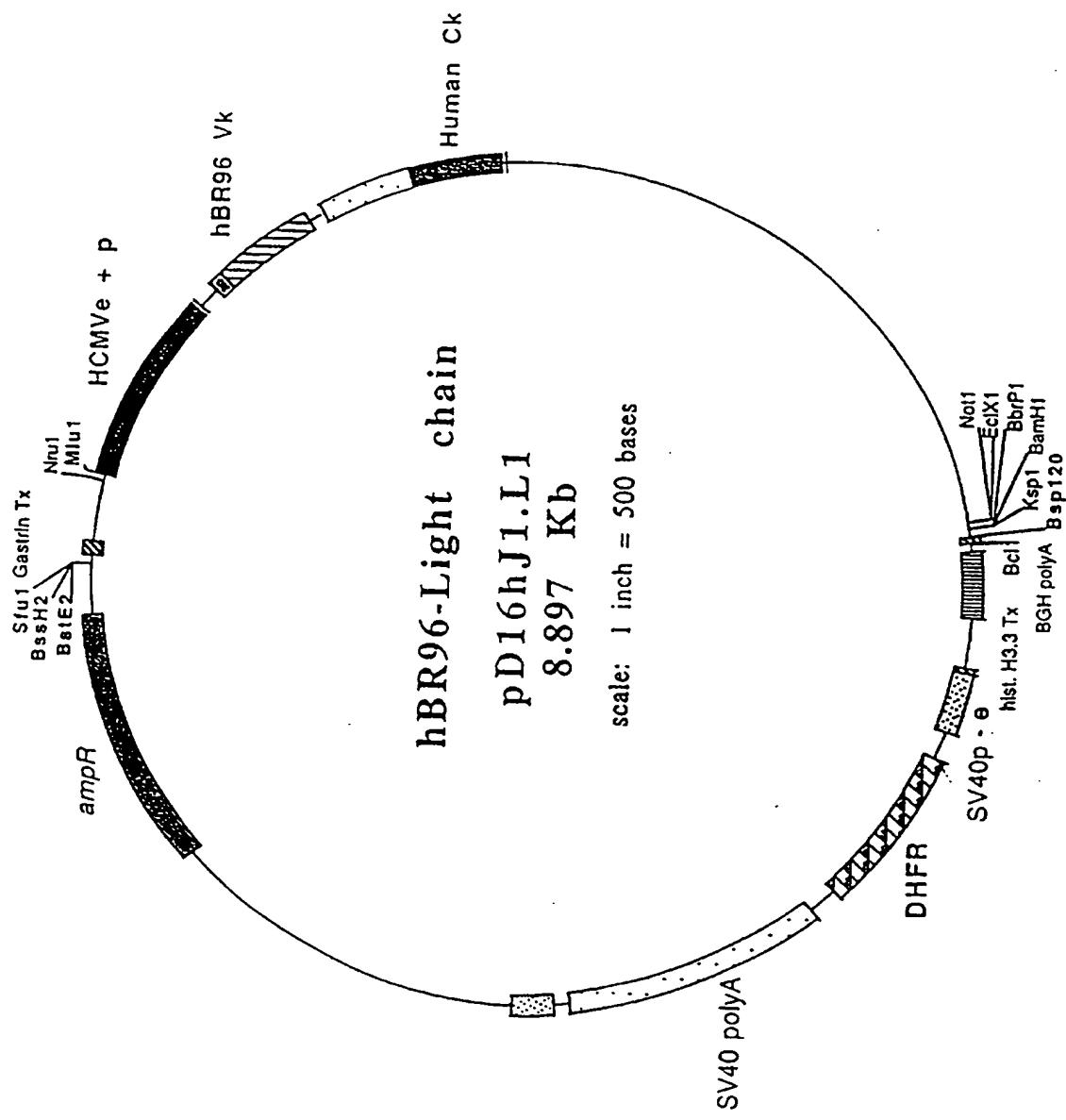


Figure 4

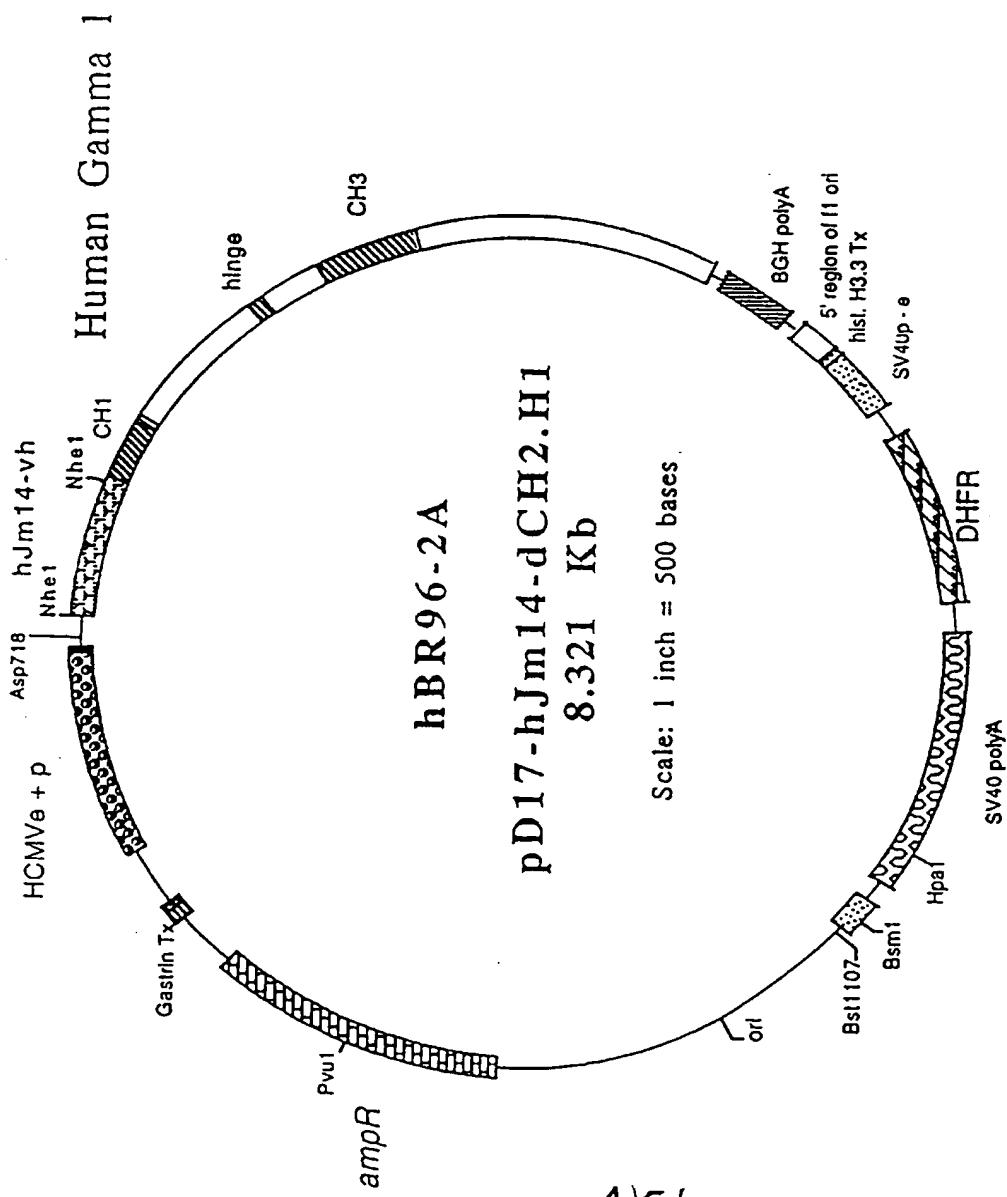


Figure 5

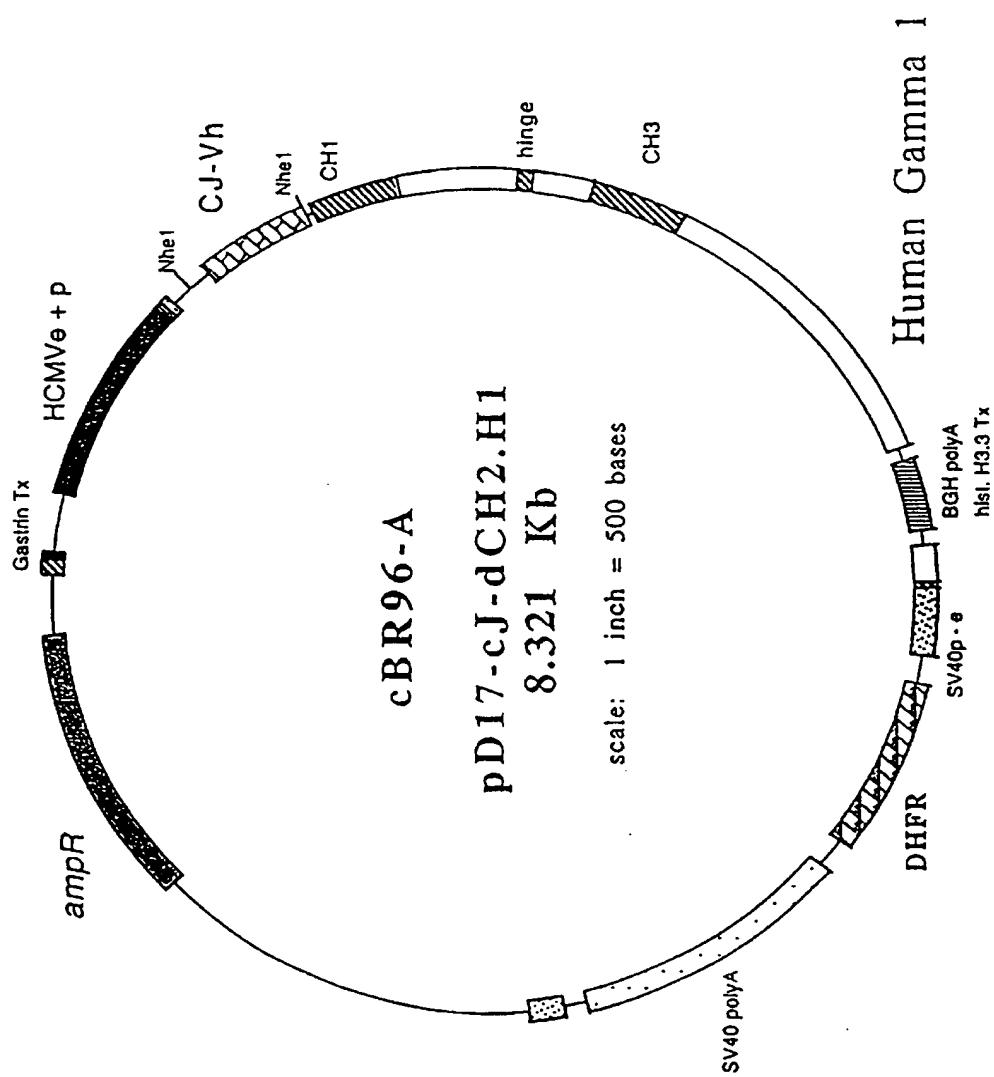
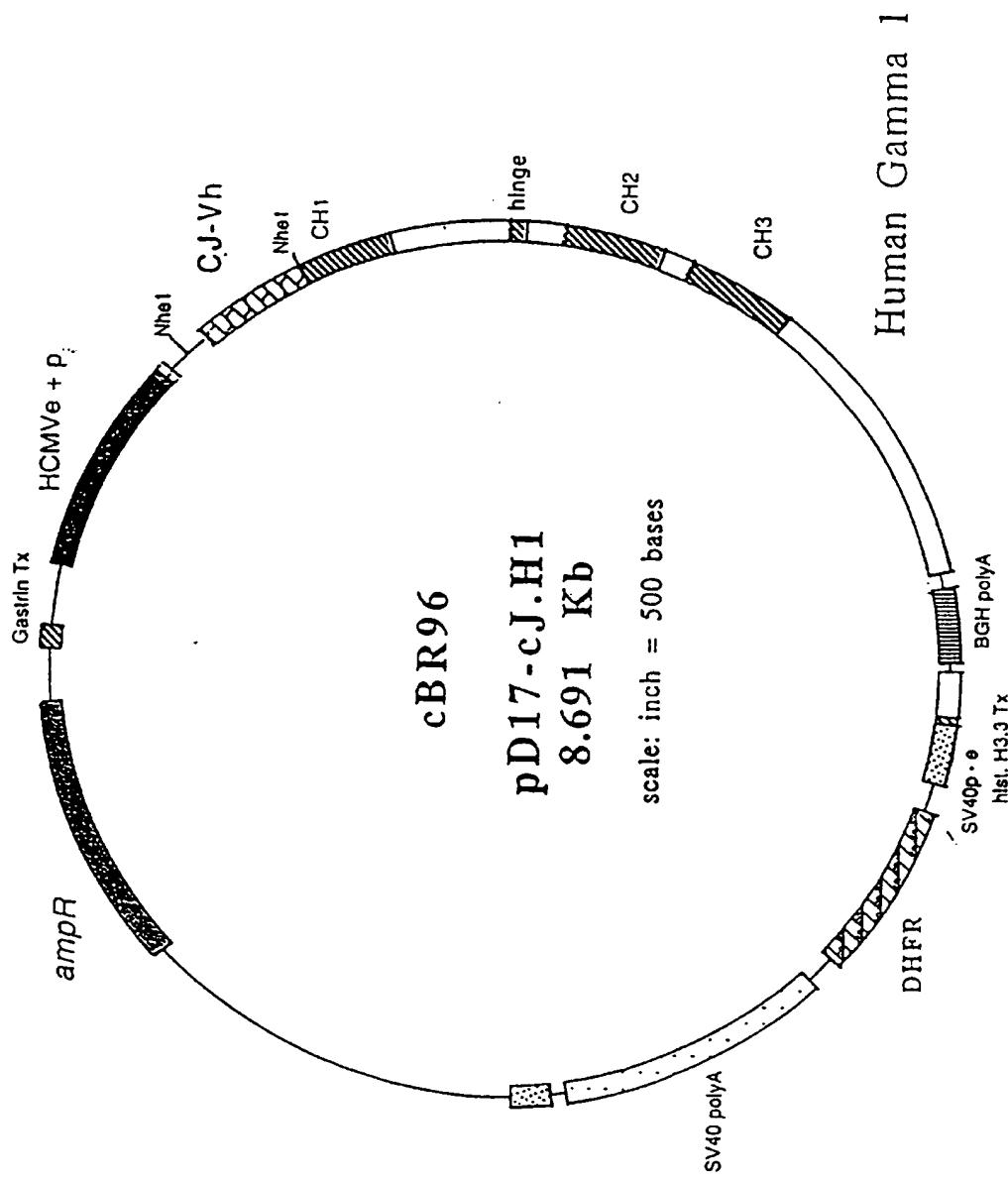
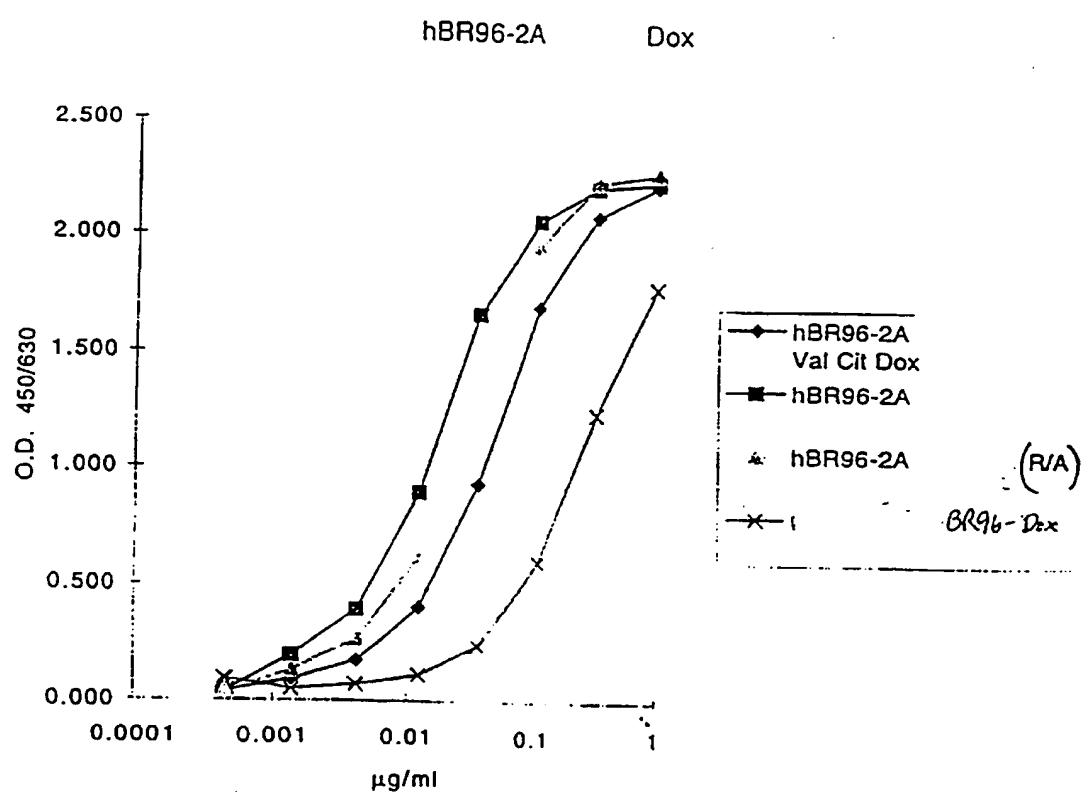


Figure 6



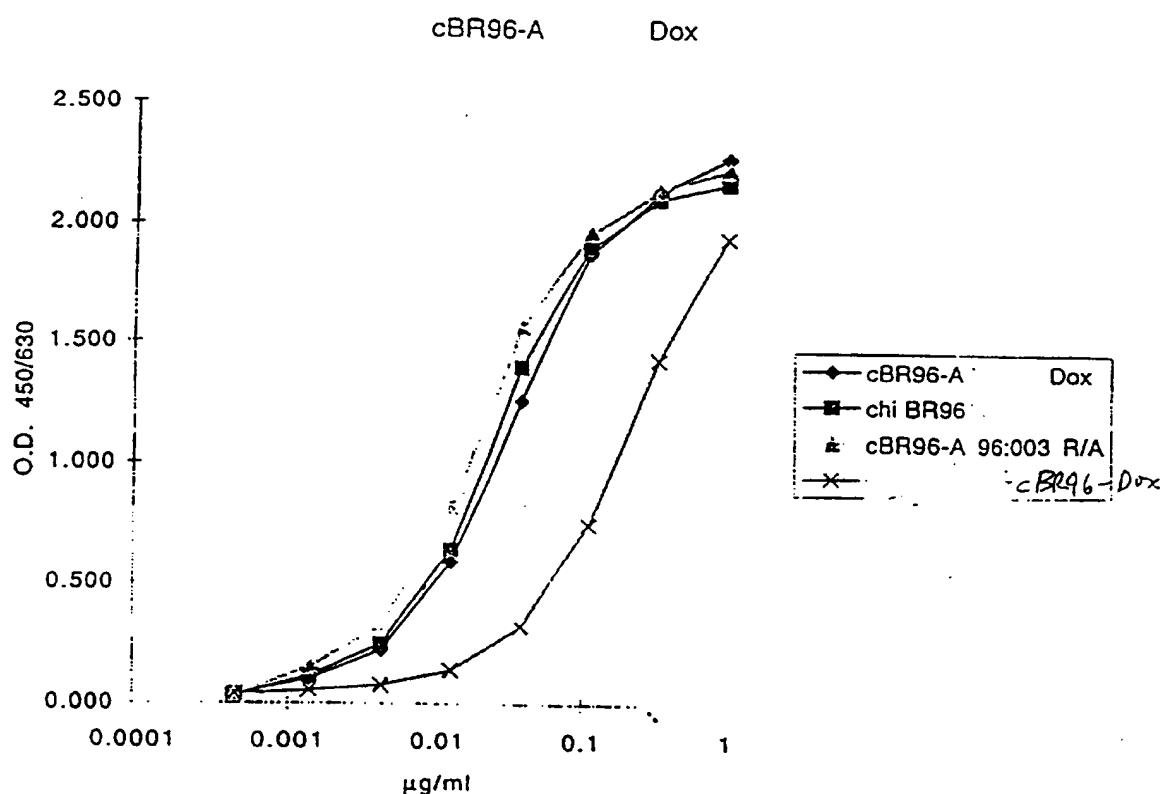
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Figure 7



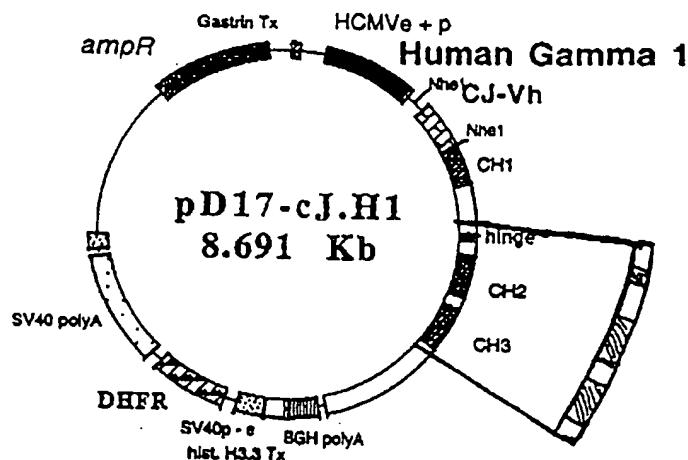
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Figure 8



8156

*A- Hinge + CH<sub>1</sub> + CH<sub>3</sub> domains were removed from L-R96 IgG1 construct by E.co I-III restriction digestion .*



*B. 2 - Hinge + CH<sub>3</sub> domains amplified by PCR from L6 IgG1 construct lacking the CH<sub>2</sub> domain .*

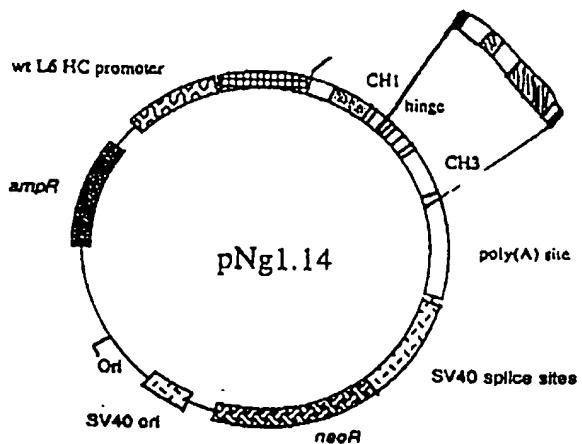


Figure 9

✓3 - Hinge +CH3 PCR fragment cloned by homologous recombination into E.co47-III site of BR96 IgG1 molecule.

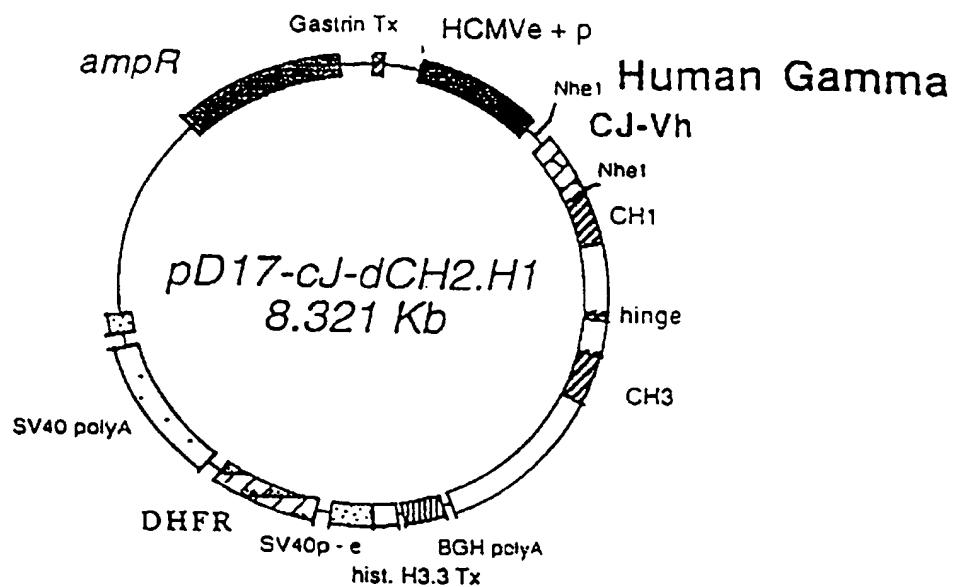
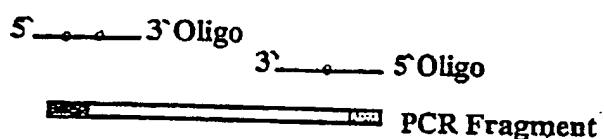


Figure 9  
(CONTINUED)

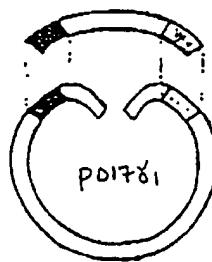
10/56

**1- Introduction of mutations by site-directed mutagenesis on double-stranded plasmid DNA.**

**A- Mutations introduced into synthetic oligonucleotides used for the PCR amplification of CH2 domain.**



**B- Plasmid DNA linearized inside CH2 domain and co-transformed with PCR fragment into competent DH5 $\alpha$ .**



**C- Cloning mediated by homologous recombination yields transformants harbouring recombinant plasmids.**

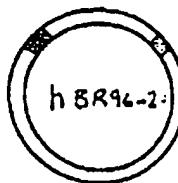
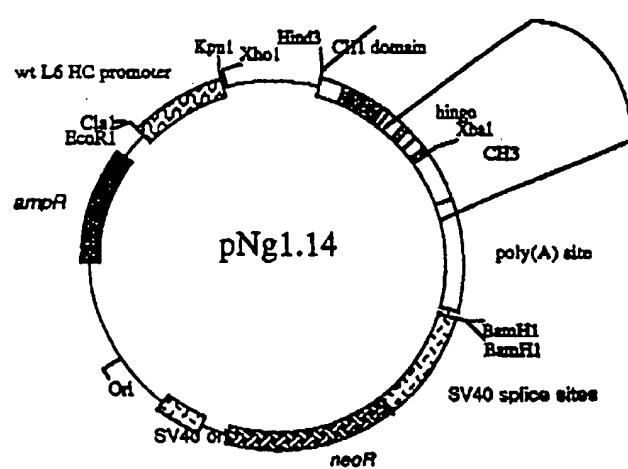


Figure 10

Figure 11



12|56

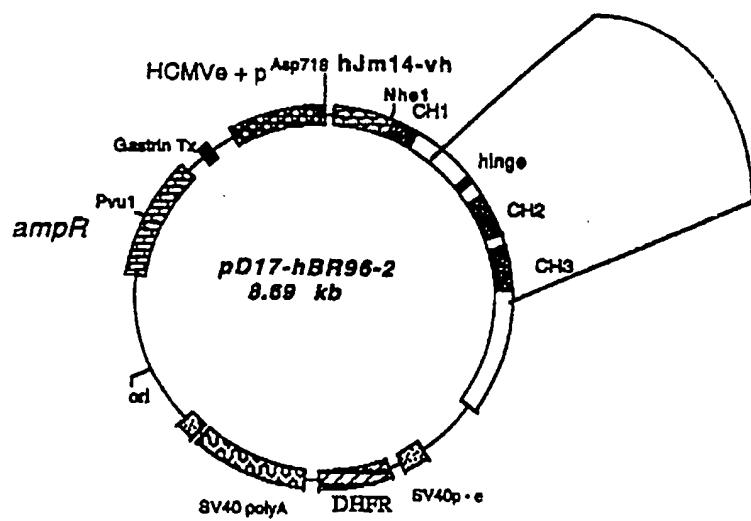


Figure 12

13156

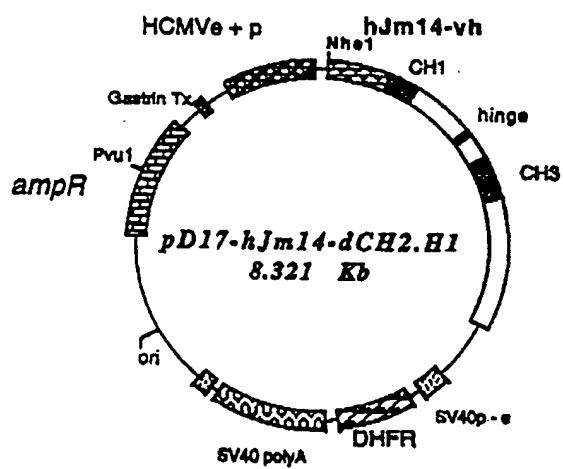


Figure 13

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## pD17-cJ-dCH2 H1

10	20	30	40	50	60	70	80	90
GACGGATCGG	GAGATCTGGT	AGGTGACCTG	AGGGCGCCG	GCTTGAATA	GGCGAGGATA	CCCTTTTT	TAATTATT	TTATTATT
CTGCTTACGCC	CTCTAGACCA	TCCACTGGAC	TCCGGCGGCC	CGAAGCTTAT	CGCGCTCATT	GGAAAAAAA	ATTAAAAAA	ATTAAAAAA
100	110	120	130	140	150	160	170	180
TTTGAGATGG	AGTTTGGCGC	ATCCCCATAG	GTGCACTCTC	AGTACACATCT	GCTCTATGC	CGCATAGTT	AGCCAGTATC	
AAACTCTAAC	TCAAACCGCG	GCTGAGGGGC	TAGGGGATAC	CAGCTGAGAG	TCACTTGTAGA	CGAGACTACG	GCCTATCAAAT	TCCGTATAG
190	200	210	220	230	240	250	260	270
TGCTCCCTGC	TGTTGTTG	GAGGTGCGT	AGTAGTGC	GAGGAAAATT	TAGCTACAA	CAAGGAAGG	CTTGACCGAC	AATTGATGA
ACGGGGACG	AACACACAAAC	CTCCAGCGAC	TCATCACGGG	CTCGTTTAA	ATTCGATGTT	GTTCCCTTC	GAACCTGCTG	TTAACGTACT
280	290	300	310	320	330	340	350	360
AGAAATCTGGT	TAGGGTTAGG	CGTTTGGC	TGCTTCGGGA	TGTPGGGCC	AGATATACGC	GTGACATG	ATTTAGTACT	AGTTTAAAT
TCTTAGACCA	ATCCCCATTC	GCAAACCGCG	ACGAAAGCGCT	ACGTGCCCCG	TCTATATGCG	CAACGTAAC	TAATTAATG	TCAATTAA
370	380	390	400	410	420	430	440	450
AGTAATCAT	TACGGGTCA	TTAGTTCA	GCCCATATAT	GGAGTTCCGC	GTACATAAAC	TTACGTTAA	TEGGCCGCT	GGCGACCGC
TCATTAAGTA	ATGCCCCAGT	ATCAACTAT	CGGGTATATA	CCCTAAGGGG	CTATGTTATG	AATGCTTAT	ACCGGGGGA	CCGACTGGCC
460	470	480	490	500	510	520	530	540
CCAACGACCC	CGGCCCATG	ACGTCAATTA	TGACGTATG	TCCCATAGTA	ACGCCATATG	GGACTTTCCA	TGAGCTCAA	TGGGTGACT
GGTTGTGGG	GGGGGGTAC	TGGCACTTAC	ATCAAGTGT	TCATATGCCA	AGTACGGCCC	CTATGACT	CAATGCGGT	AATAGCCCG
TAATGCCAT	TTGACGGGTG	AACCGTCATG	TAGTTCACAT	AGTATACGGT	TCACTGGGGG	GTAACTGCA	GTAACTGCCA	TTAACGGGC
550	560	570	580	590	600	610	620	630
ATTACGGGT	AACTGCCCCAC	TTGGCACTG	ATGACCTTAT	GGGACTTTCC	TACTGGCAG	TATAGCTACG	CGCTTATACC	ATGGGTATGC
TAATGCCAT	TTGACGGGTG	AACCGTCATG	CCCTGAATA	CCCTGAAGG	ATGACCGTC	ATATGATGTC	GGGATPATGG	TACCACTACG
640	650	660	670	680	690	700	710	720
CCTGGCATT	TGCCCACTAC	ATGACCTAT	GGGACTTTCC	TACTGGCAG	TCATCTACG	TATAGCTAC	CGCTTATACC	ATGGGTATGC
GGACCGTAA	TACGGGTATG	TACTGGGATA	CCCTGAATA	CCCTGAAGG	ATGACCGTC	ATATGATGTC	GGGATPATGG	TACCACTACG
730	740	750	760	770	780	790	800	810
GGTTGGCA	GTACATCAT	GGGGGTGGAT	AGGGGTGGA	CTCGCGGGGA	TTTCCAAAGTC	TGACGTCAT	GGGAGTTGT	
CCAAACCGT	CTAGTGTGTA	CCCGCACCA	TGCCCCCTA	AGTGGCCCT	AAAGGTCAG	AGGTCGGGTA	ACTGCGTTA	CCCTCAACAA
820	830	840	850	860	870	880	890	900
TTGGCACCA	AAATCAACGG	GACTTTCCA	AATGTCGTA	CAACTCCGCC	CCATTGACGC	AAATGGGGGG	TAGGGCGGTTA	CGGTGGGAGG
AAACCGTGT	TTTGTGTCG	CTGAAAGCTT	TTACAGCATT	GTGGGGGGG	GTAAACTGCG	TTTACCCGCC	ATCCGACAT	GCCACCCCTCC

Figure 14

15156

## pD17-cJ-dCH2 H1

910	CAGAGCTCT	920	TGGCTTAAC	930	GAGAACCCAC	940	TGGTACTGG	950	CTPATCGAA	960	TTAATAGAC	970	TCATATAGG	980	GAGACCCAAAG	
AGATATATTC	GTCCTCGAG	ACCGATGATG	CTCTGGGTG	ACGATGACC	GAATAGCTT	AATPATGCTG	AGTGTATGCC	AGTGTATGCC	AGTGTATGCC	CTCTGGGTTC	CTCTGGGTTC	CTCTGGGTTC	CTCTGGGTTC	CTCTGGGTTC		
1000	CTTGGTACCA	ATTTAAATTCG	ATATCTCTT	AGCTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGAAAT	1050	1040	1050	1060	1070	1070	1080		
GAACCATGGT	TAATTTAAC	TATAGGGAA	TCCAGAGCTC	AGAGATCTAT	TGGCCAGTAA	GCTAACCTA	GCTAACCTA	AGAACGCCG	TCTGCGGCC	GCTTGTCTAGC	GCTTGTCTAGC	GCTTGTCTAGC	GCTTGTCTAGC	GCTTGTCTAGC		
1090	1100	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200	1210	1220	1230		
CACCATGGAG	TTGTGGTTAA	GCTTGGTCCCT	TOCTTGTCCCT	TGTTTTAAAAA	GGTGTCCAGT	GTGAAGTCAA	TCTGGTGGAG	TCTGGTGGAG	TCTGGTGGAG	TCTGGTGGAG	AGAACCACTC	AGAACCACTC	AGAACCACTC	AGAACCACTC	AGAACCACTC	
GTTGACCTC	AACACCAATT	CGAACCGAGGA	AGGAACAGGA	ACAAATTTT	CCACAGGTCA	CACTCACTT	AGACCACTT	AGACCACTT	AGACCACTT	AGACCACTT	AGACCACTT	AGACCACTT	AGACCACTT	AGACCACTT	AGACCACTT	
1180	1190	1200	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320		
GCCTAGTGCA	GCCTGGGGGG	TCCCTGAGG	TCTCTGTTG	ACCTCTGGG	TTCACCTTCA	GTGACTTCA	GTGACTTCA	GTGACTTCA	GTGACTTCA	GTGACTTCA	GTGACTTCA	GTGACTTCA	GTGACTTCA	GTGACTTCA	GTGACTTCA	
CGATCACGT	CGGACCTCCC	AGGGACTTTC	AGGAGACACA	TTCGAGACCT	AGTGTGAAAT	CACTGAAAT	CACTGAAAT	CACTGAAAT	CACTGAAAT	CACTGAAAT	CACTGAAAT	CACTGAAAT	CACTGAAAT	CACTGAAAT	CACTGAAAT	
1270	1280	1290	1300	1310	1320	1330	1340	1350	1270	1280	1290	1300	1310	1320		
CCTCAGAGAA	GAGGTGGAG	TGGCTGGCAT	ACATAGTCA	AGGTGGTGT	TCTTATCACT	TATGGCTGA	TATGGCTGA	TATGGCTGA	TCTTATCACT							
GAGGTCTCTT	CTCCGACCTC	ACCCAGGGTA	TGGTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	TGTTGGGTA	
1360	1370	1380	1390	1400	1410	1420	1430	1440	1360	1370	1380	1390	1400	1410		
TCTCCAGAGA	CAATGCCAAG	AAACCCCTGT	ACCTGCAAAAT	GGGCCGTCGTG	AACTCTGAGG	ACACAGCCAT	GTATTTACTGT	GCAGAGGGCC	TCTCCAGAGA							
AGAGGTCTT	GTATCGGTTTC	TTGTGGAGCA	TGGAGGTAA	CTGGCGTTA	CTGGCGAGAC	TTGAGACTTC	TGTCGCTGTA	TGTCGCTGTA	GTGTCGCTGTA							
1450	1460	1470	1480	1490	1500	1510	1520	1530	1450	1460	1470	1480	1490	1500		
TGGACGAGC	GGCCCTGGTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCAGG	GTCTCTGTTAG	CTAGGACCAA	GGGGCCATCG	GTCTTCCCC	TGGACGAGC							
ACTGCTGCC	CGGGACCAA	CGAATGACCC	CGGTCCCTG	AGACCACTG	AGACCACTG	CAGAGACATC	GATGTTGGTT	CCCCGGTAGC	TGGACGAGC							
1540	1550	1560	1570	1580	1590	1600	1610	1620	1540	1550	1560	1570	1580	1590	1600	
TGGCACCTC	CTCCPAGAGC	ACCTCTGGG	GCACAGGGGG	CTGGGGCTGC	CTGGTCAGG	ACTACTTCCC	CGAACGGGTG	ACGGTGTGTT	TGGCACCTC							
ACCGTGGAG	GAGGTCTCTG	TGGAGACCC	CGTGTGCG	GGACCCGAGC	GGACCCGAGC	GACCACTTC	TGATGAAGGG	GTGGTGGCAC	TGGCACCTC							
1630	1640	1650	1660	1670	1680	1690	1700	1710	1630	1640	1650	1660	1670	1680	1690	
GGAAACTCAGG	CGCCCCCTRC	AGGGGGCGC	ACACCTTC	GGCTGTCCTA	CAGTCCTCA	GACTCTACTC	CCTCAGCAGC	GTGGTCACCG	GGAAACTCAGG							
CCCTGAGTCC	CGGGGGACTGG	TCGGGGACTGG	TGGGGAAAGGG	TGTGGAAAGGG	CCQACAGGAT	GTCAGGAGTC	CTGAGATGAG	GGAGGTGTCG	CCCTGAGTCC							
1720	1730	1740	1750	1760	1770	1780	1790	1800	1720	1730	1740	1750	1760	1770	1780	
TGCCCCCTCAG	CAGCTGGGG	ACCCAGACCT	ACATCTGCAA	CGTGAATCAC	AAGCCCCAGCA	ACACCAAGGT	GGACAAAGAA	GTGGGTGAGA	TGCCCCCTCAG							
ACGGGAACTC	CTCGGAACTC	TGGGGAACTC	TGGGGAACTC	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	TGTAGACGTT	

Figure 14  
(continued)

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1810	1820	1830	1840	1850	1860	1870	1880
GGCAGGCCA CGGAGGGAGG	GTGTCGGCTG	GAAGCAGGC	TCAAGCGTCC	TGCGCTGGAG	CATCCCGGCT	ATCCAGCCCC	AGTCAGGGC
CCGGTCGRTG	CCCTCCCTCG	CGCAGACGAC	CTTCGTCGGG	AGTCGGGAGG	ACGGACCTGC	GTAGCTGGGG	TGAGCTCCCG
1900	1910	1920	1930	1940	1950	1960	1970
AGCAAGGAG GCCCCGTCG	CCTCTCAC	CGGAGGCC	TCCCGCCCTC	ACTCATGGCTC	AGGGAGGGG	TCTCTGGCT	TTCCTCCAG
TCGTTCCGTC	CGGGCAGAC	GGAGAAGTGG	GCCTCGGGAG	TGAGTAGAG	TCCCTCTCCC	AGAAACCGA	AAAAGGGTC
1990	2000	2010	2020	2030	2040	2050	2060
GCTCTGGCA GGCACAGGGT	AGGTGCCCT	AACCCAGGCC	CTGACACAA	AGGGCAGGT	GCTGCGCTCA	GACCTCCAA	GAGCCATATC
CGAGACCCGT	CGTGTCCGA	TCCACGGGG	TTCGGTCCGG	GAACGTGTT	TCCCCGTCGA	CGACCCGAGT	CTCGGTTATAG
2080	2090	2100	2110	2120	2130	2140	2150
CGGAGGACC CTGCCCTGA	CCTAAGCCA	CCCCAAGGC	CAAACTCTCC	ACTCCCTCG	CTCGGACACC	TCTCTCCCTC	CCAGATCCA
CCCTCTCTGG	GAACGGGACT	GGATTGGGT	GGGGTTCCGG	GTGGAGAGG	TGAGCTGG	AGAGAGGAG	GGTCTAAGGT
2170	2180	2190	2200	2210	2220	2230	2240
GTAACCTCCA ATCTTCCTTC	TGCAAGAGCC	AAATCTTGTG	ACAAAACTCA	CACATGCCAA	CCGCGCCAGG	GTAAGCCAGC	CCAGGCCCTCG
CATGAGGGT TAGAAGAGG	ACGTCTCGGG	TTTAAACAC	TGTGTTGAGT	GTCATGGGT	GGCACGGTC	CATTCGGTCG	GGTCGGAGC
2260	2270	2280	2290	2300	2310	2320	2330
CCCTCCAGGT CAAGGGGGAA	CAGGTGCCCT	AGAGTAGGCC	GCATCCAGGG	ACACACCAAG	TGGGTACCAA	CATCTCCGGA	GCACATGGAA
GGGAGGTGA	GTTCGGCCCT	GTCCACGGGA	TCTCATCGGA	CGPAGGTCCC	1GTGTTGGTC	ACCCATGGT	CGGTGTACCT
2350	2360	2370	2380	2390	2400	2410	2420
CAGGGCCGG CTCGGCCAC	CCTCTGCCAC	GAGAGTGACC	GCCTGTACAA	CCTCTGTCAC	TACAGGGCAG	CCCCGGAGC	ACACGGTGA
GTCTCCGGCC	GAAGCCGGGT	GGAGACGGGA	CTCTCACTGG	CGACATGGGT	GGAGACAGGG	ATGTCGGTC	GTGTCACAT
2440	2450	2460	2470	2480	2490	2500	2510
CACCCCTGCC	CATCCCCGG	ATGAGGTGAC	CAAGAACAG	GTCAGCCCTGA	CCTCCCTGGT	CAAAGGGCTTC	TATTCAGCGG
GTGGGACGGG	GGTAGGGGCC	TRACTGACATG	GTTCCTGGTC	CAGTCGGACT	GTAGGGACCA	ATTCGGAAAG	TGTAGGGCA
2530	2540	2550	2560	2570	2580	2590	2600
GGAGTGGGAG AGCAATGGGC	AGCGGGAGAA	CAACTACAAG	ACCAAGCCTC	CCCTGCTGGAA	CTCCGACGGC	TCCCTCTTCC	TCTACAGCAA
CCTACCCCTC	TCGTTACCGG	TGGGCCCTT	GTGAGATGTC	TGAGTCGGAG	GGCACGACCT	GAGGAGAAGG	AGATGCTGTT
2620	2630	2640	2650	2660	2670	2680	2690
GCTCACCGTG	GACAAGAGCA	GGGGAAACGTC	TTCCTCATGCT	CGTGTATGCA	TGAGGCTCTG	CACACCACT	ACACCGAGAA
CGAGTGGCAC	CTGTTCTGGT	CCACCGTGT	CCCCTTGAG	AAAGATAGCA	GGCACTACGT	ACTCCGAGAC	GTGTTGGTGA

Figure 14  
(continued)

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2710	2720	2730	2740	2750	2760	2770	2780	2790
GAGGCTCTCC	CTGTCGCCG	GTAAATGAGT	GGGACGCCG	GCAAGCCCC	GCTCCCGGG	CTCTCGGGG	CCACAGAGGA	TGCTTGGCAC
CTCGGAGGG	GACAGAGGG	CATTTACTCA	CGCTGCGGG	CGTTCGGGG	CGAGGGCCC	GAGAGGCCA	GGTGTCTCT	ACGAACCGTG
2800	2810	2820	2830	2840	2850	2860	2870	2880
GTACCCCCCTG	TACATACTC	CGGGCGGCC	AGCATGAAA	TAAGCACCC	AGCCCTGCC	TGGGCCCTGT	CGAGACTGTG	ATGGTTCTTT
CATGGGGAC	ATGTATGAG	GGCCCCGGGG	TGTAACCTT	ATTTCGTGGG	TGCGACGGG	ACCGGGGAC	GCTCTGACAC	TACCAAGAAA
2890	2900	2910	2920	2930	2940	2950	2960	2970
CCACGGGTCA	GGCGGAGCT	GAGGCCGTAG	TGGCATGAG	GGGGAGAGC	GGGTCCACT	GTCGCCACAC	TGCCCCAGGC	TCTCAGGGTG
GGTGGCCAGT	CCGGCTCAGA	CTCCGGQACTC	ACCGTACTCC	CTCCGTCTCG	CCCTGGGTGA	CAGGGGTGTG	ACGGGTCCCG	ACAGTCCAC
2980	2990	3000	3010	3020	3030	3040	3050	3060
TCCCTGGGCC	CCCTAGGGTC	GGGGCTCGCC	AGGGGCTGCC	CTCGGAGGG	TGGGGATT	GGCAGCTCTCC	AGGAGCACCT	TGCTCGTGGG
ACGAACCCCG	GGATCTCCAC	CCCGAGTGGG	TCCCCGACGG	GAGCGTCCC	ACCCCTAAA	CGGTGCAACC	GGGGGGAGG	TGCTCGTGGG
3070	3080	3090	3100	3110	3120	3130	3140	3150
GCCCTGGCT	GGGCCACGGG	AAGCCCTAGG	AGGCCCTGGG	GACAGACACA	CGGGCCCTGG	CTCTGTAGGA	GACTGTGCTG	TCTGTGAGC
CGGGACCCGA	CCCGGTCGCC	TTCGGGATCC	TCGGGGACCC	CTGTCTGTG	GTGGGGAGG	GAGACATCT	CTGAGGGAC	AGAGACACTG
3160	3170	3180	3190	3200	3210	3220	3230	3240
GCCCCCTGTC	TCCGACCTC	CATGCCCACT	CGGGGGATG	CCTAGTCCAT	GTGGTAGGG	ACAGGGCCCTC	CCTCACCCAT	CTACCCCCAC
CGGGGACGG	AGGGCTGGAG	GTACGGGTGA	GCCCCCGTAC	GGATCAGGTA	CACGATCTCC	TGTCGGGGAG	GGATGGGTTA	GATGGGGGTC
3250	3260	3270	3280	3290	3300	3310	3320	3330
GGCACTTAC	CCTGGGTGCC	CTGCCCACTC	TGGCACCCG	ATGGGGACAC	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTCTGGAGG
CGGTGATTGG	GGACCCGCGG	GAACGGTGGG	AGGCTGGGG	TACCCCTGTG	TGGGTGAGG	CCCTGTGACG	TGAGGCCCCG	GGACACCTCC
3340	3350	3360	3370	3380	3390	3400	3410	3420
GACTGGTCA	GATGCCACCA	CACACACTCA	GCCCCAGGCC	GTTCACACAA	CCCGGCACGTG	AGGTTGGCCG	GGCACACGGC	CACCAACAC
CTGACCACT	CTACGGGTGT	GAGGTGAGT	CGGGTCTGGG	CAAGTGTGTT	GGGGCGGTGAC	TCCAACCGGC	CGGTGTCGG	GTGGTGTGTT
3430	3440	3450	3460	3470	3480	3490	3500	3510
ACACGGTCAAC	GCCTGACACA	CGGAGCCCTCA	CCCGGGGGAA	CTGCACAGCA	CCACAGCCAG	AGCAAGGTCC	TCGCACACGT	GAACACTCCT
TGTCAGGTG	CGGAGGTGTG	GCCTGCGGAT	GGGCGGGGT	GACGTGTCGT	GGTGTGTCGT	TCGTTCCAGG	AGGGTGTGCA	CTTGTTGAGGA
3520	3530	3540	3550	3560	3570	3580	3590	3600
CGGACACAGG	CCCCCACAGG	ACACCTCAAGG	CCCCAGGCC	TCTCGGGAGC	TCTCCACAT	GCTGACCTGC	TCAGACAAAC	AGTCTGTGTC
CCCCATGTC	GGGGCTCTC	GGGGTCTCCC	GGGGAGTTC	GGTGTGTCGG	AGAGGGTGA	CGACTGGACG	AGTCTGTGTC	

Figure 14  
(continued)

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## pD17-cJ-dCH2.H1

3610	TCTCACAGG	3620	3630	3640	3650	3660	3670	3680	3690
CCAGCCCTCC	GTGCCCTG	AGGCCCTGC	AGCCGCCACA	CACACACAGG	GGATCACACA	CCACGTACAG	TCCCTGGCCC	TGCCCCACTT	
GGTCGGGAGG	AGAGTGTCCC	CAAGGGAGG	TCGGGGGTGT	GTGTGTGTCC	CCTAGTGTGT	GGTCAGTGGC	AGGACCGGGG	ACGGGGTGA	
3700	3710	3720	3730	3740	3750	3760	3770	3780	
CCAGTGGCG	CCCTTCCCTG	CAAGGACGAT	CAGCTCGAC	TGTGCCCTCT	AGTGGCCAGC	CATCTGTGTT	TTCGCCCTCC	CCGTGCCCC	
GGGTCAAGGC	GGGAAGGAGC	GTCCCTGGCTA	GTGAGAGCTG	ACACGGAAAG	TCAACGGTGG	GTAGACAAAC	AACGGGAGG	GGGCACGGG	
3790	3800	3810	3820	3830	3840	3850	3860	3870	
CCGTGACCTT	GGAAAGGTGCC	ACTCCCACTG	TCCTTCCCTA	ATAAAATGAG	GAATTGGCAT	CGCATTTCT	GAGTAGGTGT	CATTCTATTC	
GGAAACTCGGA	CCTTCCACCG	TGAGGGTAC	AGGAAGGAT	TATTTTACTC	CTTTAACGTA	GGCTAACAGA	CTCATCCACA	GTAAAGATAAG	
3880	3890	3900	3910	3920	3930	3940	3950	3960	
TGGGGGGTGG	GGTGGGGGAGG	GACAGGAGG	GGAGGAGTGG	GGAGAGACAT	AGCAGGGCATG	CTGGGGATGC	GCTGGGCTCT	ATGGGCTTCCTG	
ACCCCCCACC	CCACCCCTTC	CTGTGCTTC	CCCTTCCCTAAC	CCCTTCTGTTA	TCGTCCGTAC	GACCCCTACG	CCACCCCTACG	CCACCCGAGA	
3970	3980	3990	4000	4010	4020	4030	4040	4050	
AGGGGGGAG	AACCAGCTGG	GGCTTCCCG	GGGTATCCCG	CGGGCCCTGT	AGGGGGCAT	TAAGGGCGGC	GGGTGGGGG	GTATACGGCA	
TCGGCCCTTC	TTGGTGTGAC	CCGGAGATCCC	CCATAGGGGT	GGCGGGGAGA	TGCGCGGTAA	ATTTCGGCTA	ATTTCGGCTG	CCACACCCAC	
4060	4070	4080	4090	4100	4110	4120	4130	4140	
GGGTGACCC	TACACTGCG	AGGGCCCTAG	GGCCCGCTCC	TTTGGCTTTC	TTTCCCTTCCT	TTCTCGCCAC	GTTCGCGGGG	CCCTCTAAAA	
CGCACCTGGC	ATGCTGACCG	TCGGGGGG	GGGGGGAGG	AAAGGGAAGA	AAAGGGAAGA	AAAGGGGTG	CAAGGGGGCC	GGAGAGTTT	
4150	4160	4170	4180	4190	4200	4210	4220	4230	
AAGGGAAAAA	AAGGATGCG	CTCAATTGAT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC	CCATCCCGCC	CCTAAACTCCG	CCAGGTTCCG	
TTCCCTTTT	TTCGTAACGTA	GAGTGTATCA	GTCTGTGGTA	TOAGGGCGGG	GATTGAGGG	GGATGGGG	GGATGGGG	GGGTCAAGGC	
4240	4250	4260	4270	4280	4290	4300	4310	4320	
CCCATTCCTC	GGCCCATGCG	TGACTTAATT	TTTTTATTA	TGCAAGGGCC	GAGGCCGCCT	CGGCCCGCCT	CCTATTCCTGA	AAGTAGTGAG	
GGGTAAAGGG	CGGGGTACCG	ACTGATTAA	AAAAATAAT	ACGTCTCCGG	CTCCGGGGAA	GGGGAGACT	CGATAAGGTC	TTCATCACTC	
4330	4340	4350	4360	4370	4380	4390	4400	4410	
GAGGCTTTT	TGAGGGCCPA	GGCTTPTGCA	AAAAGCTTGG	ACAGGTCAAG	GCTGGGATTT	CGGCCAAAC	TTCAGGGCAA	TCTCTAGGGTG	
CTCCGAAAAA	ACCTCCGGAT	CCGAAGACGT	TTTTCGAACC	TGTCGAGTCC	CGACGCTAA	GCGGGTTG	AACGCGCGTT	AGGATCGCAC	
4420	4430	4440	4450	4460	4470	4480	4490	4500	
AGGGCTGATA	GGATTTTATC	CCGGCTGCCCA	TCATGGTTCG	ACATTGAAC	TGCAATCGCG	CGGTGTCCCC	AAATATGGGG	ATTGGCAAGA	
TTCCGACCAT	CCTAAATAAG	GGGGGAGG	AGTACCAAGC	TGTAATCTG	ACGTAGGAGC	GGCACAGGGT	TTTAATACCCC	TAACCGTTCT	

Figure 14  
(continued)

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4510	4520	4530	4540	4550	4560	4570	4580	4590
ACGGAGACCT	ACCTGGCCT	CCGGCAGGA	ACGAGTCAA	GPACTTCCA	AGATGACCA	CAACCTTC	AGTGAGGT	AACAGATACT
TGCCCCTGG	TGGACCGGA	GGCGAGCTCT	TGCTCAAGTT	CATGAGGTT	TCTTACTCGT	GTTGGAGAAG	TGACCTTC	TTCCTGCTTAG
4600	4610	4620	4630	4640	4650	4660	4670	4680
TGGTGTATT	GGGTAGAAA	ACCTGGTCT	CCATTCTG	GAAGATCGA	CCMTTAAAGG	ACAGAAATTAA	TATAGTCTC	AGTAGAGAAC
ACCTAAATA	CCCATCTCTT	TGGACCCARGA	GGTAAGGACT	CTCTTCTAGCT	GGAAATTTC	TGTCCTPAATT	ATATCAGAG	TCTCTCTCTG
4690	4700	4710	4720	4730	4740	4750	4760	4770
TCAAAGAAC	ACCAAGAGGA	GCTCATTT	TGGCAAAAG	TPIGGATGAT	GGCTTPIAGAC	TTATIGACA	ACCGGAATTG	GCAAGTAAAG
TGTTCTGG	TGEGTCTCT	CGAGTAAAG	AACGGTTTC	AAACCTRACTA	CGGAAATTCTG	AATAACTGT	TGGCCTAAC	CGTTCTATTTC
4780	4790	4800	4810	4820	4830	4840	4850	4860
TAGACATGGT	TTCGATAGTC	GGAGGGACTT	CTGTTACCA	GGAGGCCATG	AATCAACCC	GCCACCTPAG	ACTCTTGTG	AACAGGATCA
ATCTGTACCA	AACCCATCAG	CCCTCGTAA	GACAAATGGT	CCCTCGGTAC	TTATGTTGCTC	CGGTGGGATC	TGAGAAACAC	TGTTCTCTAGT
4870	4880	4890	4900	4910	4920	4930	4940	4950
TGCGGAATT	TGAAAGTGAC	ACGTTTTCC	CAGAAATGTA	TGGGGAAA	TATAAACTTC	TCCCAAGATA	CCCAAGGCTC	CTCTCTGAGG
ACGCTCTTAA	ACTTTCACTG	TGCAAAAGG	GTCTTTRACT	AAACCCCTT	ATATTCGAA	AGGGTCTTAT	GGGTCCGAG	GAGAGACTCC
4960	4970	4980	4990	5000	5010	5020	5030	5040
TCCAGGAGGA	AAAGGCATC	AACTATAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACGG	AAGATGCTC	CAAGTCTCT	GCTCCCCCTC
AGGCTCTCT	TTCCTCGTAG	TTCATATCA	AACTTCAGAT	GCTCTTC	CTGATGTC	TTCTACCAA	GTTCAAGAGA	CGAGGGGAGG
5050	5060	5070	5080	5090	5100	5110	5120	5130
TAAGCTATG	CATTTTATA	AGACCATGCG	ACTTTGCTG	GCTTTAGTC	TCTTGTGAA	GGAACTTAC	TTCTGTGGT	TGACATAATT
ATTGATAC	CTAAAAATAT	TCTGGTACCC	TGAAAACGAC	CGAAATCTAG	AGAAACACTT	CTCTGGAT	AGAACACCAC	ACTGTATTAA
5140	5150	5160	5170	5180	5190	5200	5210	5220
GGACAAACTA	CCTAACAGAGA	TTAACGGTC	TAAGGTAAAT	ATAAAATTTC	TAAGGTATA	ATGTGTTAA	CTACTGTTTC	TAATTGTTGTC
CCCTGTTGAT	GGATGTTCT	AAATTGCGG	ATTCCATTA	TATTTTAAA	ATTCCATTT	TACACATT	TACACATT	ATTCACAAAC
5230	5240	5250	5260	5270	5280	5290	5300	5310
TGTATTTAG	ATTCACAACT	ATGGAACGTGA	TGAATGGAG	CAGCTGGAG	ATGCCCTTGA	TGAGGAAC	CTGTGTTGCT	CAGAAGAAAT
ACATAAATC	TAAGGTGCGA	TACCTGACT	ACTTACCCCT	GTCCACCT	TACGGAAATT	ACTCCCTTIG	GACAAAACGA	GTCTCTCTTA

Figure 14  
(continued)

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## pD17-cJ-dCH2.H1

5410	5420	5430	5440	5450	5460	5470	5480	5490
TTCAGAATTG	CTAAGTTTT	TGAGTOATGC	TGTGTTAGT	ATAGAAGTC	TTCGCTGCG	ACACAAAGG	AAAAAGCTGC	
AAGCTTAC	GATTAAAAA	ACTCAGTACG	ACACAAATCA	TATCTTGAG	AACGAAGGA	ACGATAATG	TGGTGTTC	TTTTTCGAGC
5500	5510	5520	5530	5540	5550	5560	5570	5580
ACTGCTTAC	AAGAAATTA	TGGAAATAA	TTCGTAAACC	TTTATAAGTA	GGCATAAAG	TTATAATCAT	ACATACATGT	TTTTTCCTAC
TGACCATATG	TTCCTTTAT	ACCTTTTAT	AAGACATTTG	AAATATTCAT	CCGTATGTC	AAATATAGTA	TTCATGACA	AAAAGAATG
5590	5600	5610	5620	5630	5640	5650	5660	5670
TCCACACAGG	CATAGAGTG	CTGCTTAA	TAACATATGT	CAAAATTGT	GTACCTTAG	CTTTTTAATT	TGTAACGGG	TTAATAAGGA
AGGTGTGTC	GTATCTCA	GACGTTAATT	ATTGATACGA	GTTTTTAACCA	CATGGAAATC	AAAAAATTA	ACATTTCCC	ATTATTCC
5680	5690	5700	5710	5720	5730	5740	5750	5760
ATATTGATG	TATAGTCCG	TGACTAGAGA	TCATAATCAG	CAATACCA	TTTGTAGGG	TTTTTACITGC	TTTAAAAAC	CTCCCCACCC
TATAAATAC	ATATCACCGA	ACTGATCT	AGTATTTAGTC	GGTATGGTG	AAACATCTCC	AAATGAACG	AAATTTTTG	GAGGGTGTGG
5770	5780	5790	5800	5810	5820	5830	5840	5850
TCCCCCTGAA	CCTGAACAT	AAATATGATG	CAATTTGT	TGTTAAACTG	TTTATTCGAG	CTTATATGCG	TTACAACATAA	AGCAATAGCA
AGGGGGACTT	GGACTTTGTA	TTTTACTAC	GTAAACACCA	ACAATTTGAC	AAATAACGT	GAATATTAC	ATGTTTAAIT	TGTTTATGTT
5860	5870	5880	5890	5900	5910	5920	5930	5940
TCACAAATT	CACAAATTAA	GCATTTTT	CACTGCATC	TAGTGTGCG	TCATCAATGT	ATCTTATCAT	GTCGGATCC	
AGTGTAA	CGTGTATT	CGTAAAATTA	GTGRCGTAAG	ATCAACACCA	ACAGGTTC	AGTAGTTACA	TAGAAATGTA	AGAACCTAGC
5950	5960	5970	5980	5990	6000	6010	6020	6030
GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCCAC	CCCAACTGT	TTTATTGCGAGC	TTATAATGGT	TACAATAAA
CGACCTRACTA	GGGGTGC	CCCTTGAGT	ACGACCTCAA	GAAGGGGTG	GGGTGAAACA	AAATACGTCG	AAATATTACCA	ATGTTTATT
6040	6050	6060	6070	6080	6090	6100	6110	6120
CGAAATGGCAT	CACAAATTTC	ACAATAAAG	CATTTTTTC	ACTGCATCT	AGTTGTGCGT	TGTCGAAACT	CATCAATGTA	TCTTTATCATG
CGTATCGTA	GTGTTAAAG	TGTTTATTC	GTAAAAAAG	TGACGTAAAG	TCAACACCA	ACAGGTGTA	GTATGTTACAT	AGAATAGTAC
6130	6140	6150	6160	6170	6180	6190	6200	6210
TCTGTATACC	GTGACCTCT	AGCTAGAGCT	TGGCGTATC	ATGGCTATAG	CTGTTTCCTG	TGTCGAAATG	TTATCCGCTC	ACATTTCCAC
AGACATATGG	CAGCTGAGA	TGGATCTCGA	ACCGCAATTAG	TACCAAGTAC	GACAAAGGAC	ACACTTAC	AAATAGGGAG	TGTTAAGGTG
6220	6230	6240	6250	6260	6270	6280	6290	6300
ACAAACATACG	AGCCGGAAGC	ATAAAGTGT	AAAGCTGGGG	TGCGCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTGTGCG	TCACGACCGC
TGTGTATGCG	TGCGCTTGC	TATTCACAT	TTCCGACCC	ACGGTAACT	CACTGGATG	AGTGTAAATTA	ACGCAACCG	AGTGAACGCC

Figure 14  
(continued)

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6310	6320	6330	6340	6350	6360	6370	6380	6390
CTTCCAGTC GGGAAACCTG	TCGTCGCCAGC	TCCATTAACTG	AATCGGCCAA	CGCGCGGGGA	GAGGGGTTT	GCCTATTGCG	CGCTCTTCGG	CGCTCTTCGG
GAAAGGTCAG	CCCTTGTGAC	ACGACGTCG	ACGTAATTCAC	GGCGCCCTT	CTTCGGCCAA	CGCATAAACCC	GGAGAAAGGC	
6400	6410	6420	6430	6440	6450	6460	6470	6480
CTTCCTGGCT	CACTGACTCG	CTGGCCTCGG	TCGTTGGCT	GGGGCGAGGG	GTATCGCTC	ACTCAAAGGC	GTAAATACGG	TTATCCACAG
GAAGGAGGCA	GTGACTGACG	GAACGAGGCC	ACGAAGGCC	CGCCGGCTCGC	CATAGTCGAG	TGAGTTCCG	CCATTATGCC	AATAGGTGTC
6490	6500	6510	6520	6530	6540	6550	6560	6570
AATCAGGGCA	TAACGCGAGA	AAGAACATGT	GAGCAAAGG	CCAGCAAAG	GGCGGAAACC	GTAAAAGGC	CGCGTTGCTG	CGGTTTTCCC
TTAGTCGCCCC	ATTCGGCTCT	TTCTGTACA	CTCGTTTCC	GGTCGTTTC	GGTCGTTTC	CGGTCCTTGG	CATTTCCTCG	GGCRAACGAC
6580	6590	6600	6610	6620	6630	6640	6650	6660
ATAGGCCTCG	CCCCCTGAC	GAGCCTCAG	AAATCGACG	CTCAAGTCAG	AGTGGGGAA	ACCCGACAGG	ACTATATAAGA	TACCAAGGGT
TATCCGAGGC	GGGGGGACTG	CTCGTAGTGT	TTTAGCTGC	GAGTCAGTC	TCCACCGCTT	TGGCTGTCC	TCATATTCT	ATGGTCGCCA
6670	6680	6690	6700	6710	6720	6730	6740	6750
TTCCCCCTGG	AAAGTCCTC	GTGGGCTCTC	CTGTTCCGAC	CTGTCGCTT	ACGGGATACC	TCTCCGCCCT	TCTCCCTTCG	CGAAGCGTGG
AAGGGGGAGCC	TTCGAGGGAG	CACGGAGAG	GACAAGGCTG	GGACGGGGAA	TGGCCTATGG	ACAGGGGAA	AGAGGAAGC	CTCTCGCACC
6760	6770	6780	6790	6800	6810	6820	6830	6840
CGCTTTCTCA	ATGCTCACCG	TGTAGGTATC	TOAGTTGGGT	GGAGGTGGT	CGCTCCAAAGC	TGGCTGTGT	GCACGAACCC	CCGGTTCAGC
GGCAAAGAGGT	TACGAGTGGG	ACATCCATAG	AGTCAAAGCCA	CATCCAGCAA	GGGAGGTTCG	ACCCGACACA	CGTCCTTGGG	GGCGAAGTGG
6850	6860	6870	6880	6890	6900	6910	6920	6930
CGACCCGCTG	CGCTTATCC	GGTAACATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATGCCCACT	GGCAGCAGCC	ACTGGTAACA
GGCTGGGAGC	GGGGAAATGG	CCATTGATAG	CAGAACTCAG	GTGGGGCAT	TCTGTGTGA	ATAGGGTGA	CCGTCGTGG	TGACCATTTG
6940	6950	6960	6970	6980	6990	7000	7010	7020
GGATTAGCAG	AGCGAGGTAT	GTAGGGGGTG	CTACAGAGTT	CTTGAAGTGG	TGCCCCTAAC	ACGGCTACAC	TAGAAGGACA	GTATTGGATA
CCTAATCGTC	TGGCTCATA	CATCGCCAC	GTGTCCTCAA	GAACCTCAAC	ACCGGATGTA	TGCCGATGTG	ATCTTCCTGT	CATAAACCAT
7030	7040	7050	7060	7070	7080	7090	7100	7110
TCTGGGCTCT	GCTGAAGCCA	GTTCACCTTCG	GAAGAAAGAGT	TGGTAGCTT	TGATCCGGAA	AACAAACCC	CGCGTGGTAGC	GGTGGTTTT
AGACGGAGGA	CGACTTCGGT	CAATGAAAGC	CTTTCCTCA	ACCATCGAGA	ACPGGGCGT	TGGTTGGTG	GGGACCATCG	CCACCCAAA
7120	7130	7140	7150	7160	7170	7180	7190	7200
TGTTGCAA	GCAGCAGATT	ACGGCGAGAA	AAAAGGATC	TGAGAAAGAT	CCPTTGACT	TTTCTACGGG	GGCTGAGGCT	CAGTGGAAAG
AACAAACGTT	CGTCGCTAA	TCGGCTCTT	TTTTCCTAG	AGTCTCTTA	GGAAACTGAA	AAAGATGCCA	GTACCCCTGC	

Figure 14  
(continued)

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## pD17-cJ-dCH2.H1

7210 AAAACTCACG 7220 TTAAGGGATT 7230 TGGGTATGA 7240 GATTATCAA 7250 AAGGAACTTC 7260 ACCTAGATCC 7270 TTTTAATTA 7280 AAAATGAAT 7290 TTTTGAGTGC AATTCCCTAA ACCAGTACT 7240 CTAAATAGTTT 7250 TCCCTAGAG 7260 TGGATCTGAA 7270 AAAATTAAAT 7280 TTTACTCA AAATTAGTT  
 7300 TCTAATGAT 7310 ATATGAGTAA 7320 ACAGCTTACCA 7330 ATGCTTAAATC 7340 AGTGGGCCAC 7350 CTATCTAGC 7360 GATCTGCTCA 7370 TTTCGTTCAT  
 AGATTTCATA TATATCTATT TGACCAAGAC 7320 TGCTATGGT 7330 TACGAATTAG 7340 TCACTCGTG 7350 GATAAGTCG 7360 CTAGACAGT AAGCAAGTA  
 7390 CCGTAGTGC 7400 CCGACTCCC 7410 GTCGTGTAGA 7420 TAACCTAGAT 7430 ACGGGAGGC 7440 TTACCATGTS 7450 GCCCCAGTC 7460 TCCAATGATA 7470 CCGGAGAACC  
 GGTATCAACG 7400 GACTGAGGG 7410 CAGGCACTCT 7420 ATTCTAGCTA 7430 TGGCCCTCCG 7440 AATGGTAGAC 7450 CGGGGTCAAG 7460 ACGTTACTAT 7470 GGGGCTCTGG  
 7480 CACGGCTAAC 7490 GGTCTCAGT 7500 TTATGAGCAA 7510 TAACCGAGCC 7520 AGCCGGAGG 7530 GCGGAGGCCA 7540 GAAGTGGTCC 7550 TGGCAACTTTA 7560 TCCGGCTTCCA  
 GTGGGAGTGG 7500 CCGAGGTCTT 7510 ATTCTGGTCC 7520 TCGGCCTTCG 7530 CGGCTCGGGT 7540 CTTTCACCGG 7550 AGGGCGAGGT  
 7570 7580 7590 7600 7610 7620 7630 7640 7650  
 TCCAGTCTAT TATTTGTC 7570 CGGGAGAGT 7580 GAGTGTAGT 7590 TTTCGCAATT 7600 AATGTTGTC 7610 GCAAGCTTGT 7620 TGGCACTTGT 7630 ACAGGCATCG  
 AGGTCAAGATA 7570 ATTACAAAG 7580 GCCCCTTGAT 7590 CTCACTCATC 7600 AAGGGSTCAA 7610 TTATCTAACG 7620 ACGTGAACGA 7630 AGGGCGAGGT  
 7660 7670 7680 7690 7700 7710 7720 7730 7740  
 TGGTGTACG CTGCTCGTT 7670 GGTATGCTT 7680 CATTCTAGTC 7690 CGGTTCCTAA 7700 CGATCAAGGC 7710 GAGTTCATC 7720 ATCCCCATG 7730 TTGTCACAAA  
 ACCACAGTCG GAGCAGCAA 7670 CCATACGAA 7680 GTAGTCGAG 7690 GCGCAAGGGT 7700 GCTAGTCG 7710 CGTTCACATC 7720 TAGGGGTAC 7730 AACACGTTT  
 7750 7760 7770 7780 7790 7800 7810 7820 7830  
 AACGGGTTAG CTCTTCGCGT 7760 CCTCGATCG 7770 TTGTGAGAAG 7780 TAAGTGGCC 7790 GCAGTGTAT 7800 CACTCTGGT 7810 TATGGCAGCA 7820 CTGCAATT  
 TTGGCCAAATC GAGGAAGCCA 7760 CGAGGCTAGC 7770 AACGTCCTTC 7780 ATTCAACGG 7790 CGTCACAAAT 7800 GTGAGTACCA 7810 ATACCGTGT 7820 GACGTATTAA  
 7840 7850 7860 7870 7880 7890 7900 7910 7920  
 CTCTTACTGT CATGCCATCC 7850 GTAAAGATGCT 7860 TTTCCTGTGAC 7870 TGGTGTAGTAC 7880 TCAACCAAGT 7890 CATTCTGAGA 7900 ATAGTGTATG 7910 CGGGCACCGA  
 GAGAATGACA 7850 GTACGGTAGG 7860 CATTCTACGA 7870 AAAGACACTG 7880 ACCACTCTG 7890 AGTTCGTTCA 7900 TATCACATAC 7910 GCGCGCTGGCT  
 7930 7940 7950 7960 7970 7980 7990 8000 8010  
 GTTGCTCTTG CCCGGCTGTC 7940 ATACGGATA 7950 ACATAGCAGA 7960 ACTTTAAAAG 7970 TGCTTCAT 7980 TCGAAAACGT 7990 8000  
 CAACGAGAAC 7940 GGGCCGAGT 7950 TATGCCCTAT 7960 TATGGCGGG 7970 TGTATCGTCT 7980 ACAGTGTATA 8000 ACCTTTTCGA 7990 AGAAGCCCCG  
 8020 8030 8040 8050 8060 8070 8080 8090 8100  
 GAAAACCTC AAGGATCTA 8020 CGCGCTGTTGA 8030 GATCGAGTC 8040 ACTCGTGCAC 8050 CCAACTGATC 8060 TTCAGGCTCT 8070 TTTACTTTCA 8080 8090  
 CTTTTGAGAG 8020 TCTCTAGAAT 8030 GGGGACAACT 8040 CTAGGTCAAG 8050 CTACATTGGG 8060 TGGGACGGTG 8070 AAAGCTGACTAG 8080 AAATGAAGT

Figure 14  
(continued)

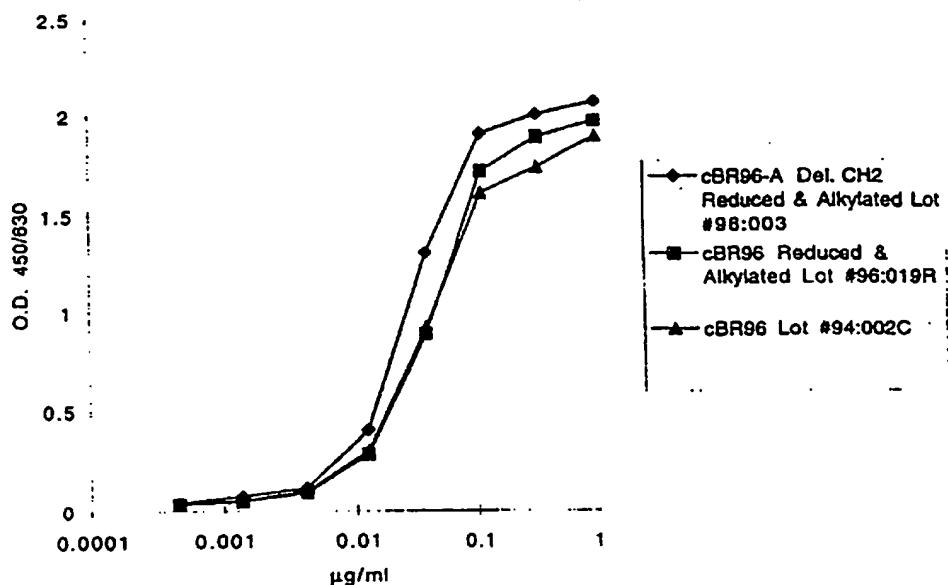
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8110	8120	8130	8140	8150	8160	8170	8180	8190	
CCAGGTTTC	TGGGTGCGCA	AAACAGCA	GGCAAAATGC	GGAAATAGG	CGCACCGGA	ATGTGAATA	CTCATACTCT		
GGTCGAAAG	ACCCACTCGT	TTTGTGCTT	CCGGTTTACG	GGTTTTCC	GCTGTGCCCT	TACAACCTAT	GAGTATGAGA		
8200	8210	8220	8230	8240	8250	8260	8270	8280	
TCCCTTTCA	ATATTTGAA	AGCCATTATC	ACGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	
AGGAAAGT	TATAATRACT	TCGTAAATAG	TCCCAATAAC	AGAGTACTCG	CCATGTATA	AACCTACATA	AACTACATA	AACTACATA	TTGTGTTATC
8290	8300	8310	8320	8330					
GGGTTCCGG	CACATTCCC	CGAAAGTC	CACCTGACGT	C					
CCCCAAGGSC	GTGTAAAGGGS	GCTTTTACG	GTGGACTGCA	G					

Figure 14  
(continued)

24150

Comparison of whole chiBR96 and  
deleted CH2 chiBR96 on Ley/K ELISA



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hBR96-2B: L235 to A235 and G237 to A237

hBR96-2C: E318 to S318, K320 to S320, and K322 to S322

hBR96-2D: P331 to A331

hBR96-2E: L235 to A235, G237 to A237, E318 to S318, K320 to S320, and K322 to S322

hBR96-2F: L235 to A235, G237 to A237, and P331 to A331

hBR96-2G: E318 to S318, K320 to S320, K322 to S322, and P331 to A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320, K322 to S322, and P331 to A331

Figure 16

26)56

FIGURE 17

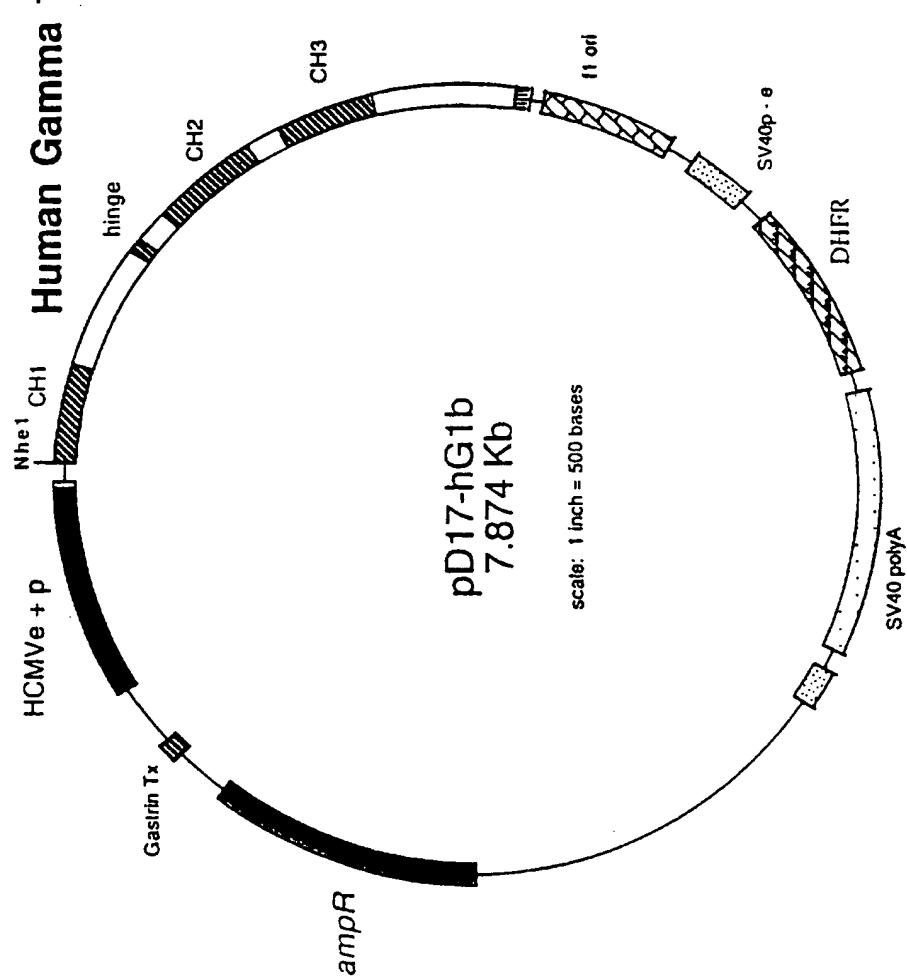


FIGURE 18A

1 GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC  
51 GGTCAATCGA TTGGAATTCT TGCAGGCCGCT TGCTAGCCAC CATGGAGTTG  
101 TGGTTAAGCT TGGTCTTCCT TGTCCCTGTT TTAAAAGGTG TCCAGTGTGA  
151 AGTGCAACTG GTGGAGTCTG GGGGAGGCTT AGTGCAGCCT GGAGGGTCCC  
201 TGCAGCTTTC CTGTGCTGCA TCTGGATTCC CGTTCACTGA CTATTACATG  
251 TATTGGGTTTC GCCAGGCTCC AGGCAAGGGA CTGGAGTGGG TCTCATAACAT  
301 TAGTCAAGAT GGTGATATAA CCGACTATGC AGACTCCGTA AAGGGTCGAT  
351 TCACCATCTC CAGAGACAAT GCAAAGAACCA GCCTGTACCT GCAAATGAAC  
401 AGCCTGAGGG ACGAGGACAC AGCCGTGTAT TACTGTGCAA GAGGCCTGGC  
451 GGACGGGGCC TGGTTTGCTT ACTGGGGCCA AGGGACTCTG GTCACGGTCT  
501 CTTCCGCTAG CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCCTCC  
551 AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA  
601 CTTCCCCGAA CCGGTGACGG TGCTGTGGAA CTCAGGCCCTG CTGACCAGCG  
651 GCGTGCACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC  
701 AGCAGCGTGG TCACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT  
751 CTGCAACGTG AATCACAAAGC CCAGCAACAC CAAGGTGGAC AAGAAAGTTG  
801 GTGAGAGGCC AGCACAGGGA GGGAGGGTGT CTGCTGGAAG CCAGGCTCAG  
851 CGCTCCTGCC TGGACGCATC CCGGCTATGC AGCCCCAGTC CAGGGCAGCA  
901 AGGCAGGCCCG CGTCTGCCTC TTCACCCGGA GGCCTCTGCC CGCCCCACTC  
951 ATGCTCAGGG AGAGGGTCTT CTGGCTTTTT CCCCAGGCTC TGGGCAGGCA  
1001 CAGGCTAGGT GCCCCTAACCC CAGGCCCTGC ACACAAAGGG GCAGGTGCTG  
1051 GGCTCAGACC TGCCAAGAGC CATATCCGGG AGGACCCCTGC CCCTGACCTA  
1101 AGCCCCACCCCA AAAGGCCAAA CTCTCCACTC CCTCAGCTCG GACACCTTCT  
1151 CTCCTCCCAG ATTCCAGTAA CTCCCAATCT TCTCTCTGCA GAGCCCAAAT  
1201 CTTGTGACAA AACTCACACA TGCCCACCGT GCCCAGGTAA GCCAGCCCAG  
1251 GCCTCGCCCT CCAGCTCAAG GCGGGACAGG TCCCCTAGAG TAGCCTGCAT  
1301 CCAGGGACAG GCCCCAGCCG GGTGCTGACA CGTCCACCTC CATCTCTTCC

1351 TCAGCACCTG AACTC<sup>335</sup>GG G<sup>337</sup>CGTCA GTCTTCCTCT TCCCCCCC  
 1401 ACCCAAGGAC ACCCTCATGA TCTCCGGAC CCCTGAGGTC ACATGCGTGG  
 1451 TGGTGGACGT GAGCCACGAA GACCTGAGG TCAAGTTCAA CTGGTACGTG  
 1501 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA  
 1551 CAACAGCACG TACCGTGTGG TCAGCGTC<sup>318</sup> CACCGTC<sup>320</sup>CTG CACCAAGGACT  
 1601 GGCTGAATGG CAAG<sup>331</sup>GTAC AAGTGC<sup>322</sup>AGG TCTCCAACAA AGCCCTCCCA  
 1651 GC<sup>331</sup>CCATCG AGAAAACCAT CTCCAAGCC AAAGGTGGGA CCCGTGGGGT  
 1701 GCGAGGGCCA CATGGACAGA GGCGGGCTCG GCCCACCC<sup>323</sup>TC TGCCCTGAGA  
 1751 GTGACCGCTG TACCAACCTC TGTCCTACA GGGCAGCCCC GAGAACCACA  
 1801 GGTGTACACC CTGCCCCAT CCCGGGATGA GCTGACCAAG AACCAAGGTCA  
 1851 GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG  
 1901 TGGGAGAGCA ATGGGCAGCC GGAGAACAAAC TACAAGACCA CGCCTCCCGT  
 1951 GCTGGACTCC GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA  
 2001 AGAGCAGGTG GCAGCAGGGG AACGTC<sup>324</sup>TCT CATGCTCCGT GATGCATGAG  
 2051 GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA  
 2101 ATGAGTGC<sup>325</sup>GA CGGCCGGCAA GCCCGC<sup>326</sup>TC CCCGGCTCT CGCGGT<sup>327</sup>CGCA  
 2151 CGAGGATGCT TGGCACGTAC CCCCTGTACA TACTTCCCGG GCGCCCAGCA  
 2201 TGGAAATAAA GCACCCAGCG CTGCCCTGGG CCCCTGCGAG ACTGTGATGG  
 2251 TTCTTCCAC GGGTCAGGCC GAGTCTGAGG CCTGAGTGGC ATGAGGGAGG  
 2301 CAGAGCGGGT CCCACTGTCC CCACACTGGC CCAGGCTGTG CAGGTGTGCC  
 2351 TGGGCCCCCT AGGGTGGGGC TCAGCCAGGG GCTGCCCTCG GCAGGGTGGG  
 2401 GGATTGCCA GCGTGGCCCT CCCTCCAGCA GCACCTGCC<sup>328</sup> TGGGCTGGGC  
 2451 CACGGGAAGC CCTAGGAGCC CCTGGGGACA GACACACAGC CCCTGCCTCT  
 2501 GTAGGAGACT GTCCTGTTCT GTGAGCGCCC CTGTCCTCCC GACCTCCATG  
 2551 CCCACTCGGG GGCATGCCTA GTCCATGTGC GTAGGGACAG GCCCTCCCTC  
 2601 ACCCATCTAC CCCCACGGCA CTAACCC<sup>329</sup>TG GCTGCCCTGC CCAGCCTCGC  
 2651 ACCCGCATGG GGACACAAACC GACTCCGGGG ACATGCACTC TCGGGCCCTG  
 2701 TGGAGGGACT GGTGCAGATG CCCACACACA CACTCAGCCC AGACCCGTTTC  
 2751 AACAAACCCC GCACTGAGGT TGGCCGGCCA CACGGCCACC ACACACACAC  
 2801 GTGCACGCCT CACACACGG<sup>330</sup> GA GCCTCACCCG GGCGAACTGC ACAGCACCC<sup>331</sup>A

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2851 GACCAGAGCA AGG CCTCGC ACACGTGAAC ACTCCTCGGA CACAGGCCCC  
2901 CACGAGCCCC ACGCGGCACC TCAAGGCCA CGAGCCTCTC GGCAGCTTCT  
2951 CCACATGCTG ACCTGCTCAG ACAAAACCCAG CCCTCCTCTC ACAAGGGTGC  
3001 CCCTGCAGCC GCCACACACA CACAGGGAT CACACACCAC GTCACGTCCC  
3051 TGGCCCTGGC CCACTTCCA GTGCCGCCCT TCCCTGCAGG ACGGATCAGC  
3101 CTCGACTGTG CCTTCTAGTT GCCAGCCATC TGGTGTTCGC CCCTCCCCG  
3151 TGCCCTCCTT GACCCCTGGAA GGTGCCACTC CCACTGTCCCT TTCCCTAATAA  
3201 AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCAATT CTATTCTGGG  
3251 GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA  
3301 GGCATGCTGG GGATGCGGTG GGCTCTATGG CTTCTGAGGC GGAAAGAAC  
3351 AGCTGGGGCT CTAGGGGGTA TCCCCACGCG CCCTGTAGCG GCGCATTAAAG  
3401 CGCGGGGGGT GTGGTGGTTA CGCCGAGCGT GACCGCTACA CTTGCCAGCG  
3451 CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCTTTCT CGCCACGTT  
3501 GCCGGGCCTC TCAAAAAGG GAAAAAAAGC ATGCATCTCA ATTAGTCAGC  
3551 AACCATAGTC CCGCCCCCTAA CTCCGCCAT CCCGCCCTA ACTCCGCCA  
3601 GTTCCGCCA TTCTCCGCC CATGGCTGAC TAATTTTTT TATTTATGCA  
3651 GAGGCCGAGG CCGCCTCGGC CTCTGAGCTA TTCCAGAAGT AGTGAGGAGG  
3701 CTTTTTGGA GGCCTAGGCT TTTGCAAAAA GCTTGGACAG CTCAGGGCTG  
3751 CGATTCGCG CCAAACCTGA CGGCAATCCT AGCGTGAAGG CTGGTAGGAT  
3801 TTTATCCCCG CTGCCATCAT GGTCGACCA TTGAAC TGCA TCGTCGCCGT  
3851 GTCCCCAAAT ATGGGGATTG GCAAGAACGG AGACCTACCC TGGCCTCCGC  
3901 TCAGGAACGA GTTCAAGTAC TTCCAAAGAA TGACCACAAAC CTCTTCAGTG  
3951 GAAGGTAAAC AGAATCTGGT GATTATGGGT AGGAAAACCT GGTTCTCCAT  
4001 TCCTGAGAAG AATCGACCTT TAAAGGACAG AATTAATATA GTTCTCAGTA  
4051 GAGAACTCAA AGAACCAACCA CGAGGGAGCTC ATTTTCTTGC CAAAAGTTG  
4101 GATGATGCCT TAAGACTTAT TGAACAAACCG GAATTGGCAA GTAAAGTACA  
4151 CATGGTTGG ATAGTCGGAG CGAGTTCTGT TTACCCAGGAA GCCATGAATC  
4201 AACCAAGGCCA CCTTAGACTC TTTGTGACAA GGATCATGCA GGAATTGAA  
4251 AGTGACACGT TTTCCCAGA AATTGATTG GGGAAATATA AACCTCTCCC  
4301 AGAATACCCA GGCCTCCTCT CTGAGGTCCA GGAGGAAAAA GGCATCAAGT

4351 ATAAGTTGA AGTCTACGAG AAGAAAGACT AACAGGAAGA TGCTTCAG  
4401 TTCTCTGCTC CCCTCTAAA GCTATGCATT TTTATAAGAC CATGGGACTT  
4451 TTGCTGGCTT TAGATCTCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC  
4501 ATAATTGGAC AAACTACCTA CAGAGATTAA AAGCTCTAAG GTAAATATAA  
4551 AATTTTAAG TGTATAATGT GTTAAACTAC TGATTCTAAT TGTGTTGTGA  
4601 TTTTAGATTC CAACCTATGG AACTGATGAA TGGGAGCAGT GGTGGAATGC  
4651 CTTTAATGAG GAAAACCTGT TTTGCTCAGA AGAAATGCCA TCTAGTGTG  
4701 ATGAGGCTAC TGCTGACTCT CAACATTCTA CTCCCTCCAAA AAAGAAGAGA  
4751 AAGGTAGAAG ACCCCAAAGGA CTTTCCTTCA GAATTGCTAA GTTTTTGAG  
4801 TCATGCTGTG TTTAGTAATA GAACTCTTGC TTGCTTGCT ATTTACACCA  
4851 CAAAGGAAAA AGCTGCACTG CTATACAAGA AAATTATGGA AAAATATTCT  
4901 GTAACCTTTA TAAGTAGGCA TAACAGTTAT AATCATAACA TACTGTTTT  
4951 TCTTACTCCA CACAGGCATA GAGTGTCTGC TATTAATAAC TATGCTCAA  
5001 AATTGTGTAC CTTTAGCTT TTAATTGTA AAGGGGTTAA TAAGGAATAT  
5051 TTGATGTATA GTGCCTTGAC TAGAGATCAT AATCAGCCAT ACCACATTG  
5101 TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG  
5151 AAACATAAAA TGAATGCAAT TGTTGTTGTT AACTTGTTA TTGCAGCTTA  
5201 TAATGGTTAC AAATAAGCA ATAGCATCAC AAATTCACA AATAAGCAT  
5251 TTTTTCACT GCATTCTAGT TGTGGTTGT CCAAACTCAT CAATGTATCT  
5301 TATCATGTCT GGATCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT  
5351 GGAGTTCTTC GCCCACCCCA ACTTGTATT TGCAGCTTAT AATGGTTACA  
5401 AATAAAGCAA TAGCATCACA AATTCACAA ATAAAGCATT TTTTCACTG  
5451 CATTCTAGTT GTGGTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG  
5501 TATACCGTCG ACCTCTAGCT AGAGCTTGGC GTAATCATGG TCATAGCTGT  
5551 TTCCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAA CATACTGAGCC  
5601 GGAAGCATAA AGTGTAAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC  
5651 ATTAATTGCG TTGCGCTCAC TGCCCGCTT CCAGTCGGGA AACCTGTCGT  
5701 GCCAGCTGCA TTAATGAATC GGCCAAACCGG CGGGGAGAGG CGGTTTGCCT  
5751 ATTGGGCGCT CTTCCGCTTC CTCCGCTCACT GACTCGCTGC GCTCGGTGCGT  
5801 TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA ATACGGTTAT

5351 CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG  
5901 CAAAAGGCCA GGAACCGTAA AAAGGCCCGG TTGCTGGCGT TTTTCCATAG  
5951 GCTCCGCCCG CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT  
6001 GGCAGAAACCC GACAGGACTA TAAAGATACC AGGCCTTCC CCCTGGAAGC  
6051 TCCCTCGTGC GCTCTCCTGT TCCGACCCCTG CCGCTTACCG GATAACCTGTC  
6101 CGCCTTCTC CTTTCGGAA GCGTGGCGCT TTCTCAATGC TCACGCTGTA  
6151 GGTATCTCAG TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC  
6201 GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT  
6251 TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG  
6301 GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG  
6351 AAGTGGTGGC CTAACTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG  
6401 CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT  
6451 CCGGCAAACA AACCAACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG  
6501 CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC  
6551 TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTGG  
6601 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAAAAA  
6651 TGAAGTTTA AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG  
6701 TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTC  
6751 GTTCATCCAT AGTTGCCTGA CTCCCCGTG TGTTAGATAAC TACGATAACGG  
6801 GAGGGCTTAC CATCTGGCCC CAGTGTGCA ATGATAACCGC GAGACCCACG  
6851 CTCACCGGCT CCAGATTTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG  
6901 AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT  
6951 TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTGCACAA  
7001 CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG TCGTTGGTA  
7051 TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT TACATGATCC  
7101 CCCATGTTGT GCAAAAAAGC GGTTAGCTCC TTGGTGCCTC CGATCGTTGT  
7151 CAGAAGTAAG TTGGCCCGCAG TGTTATCACT CATGGTTATG GCAGCACTGC  
7201 ATAATTCTCT TACTGTGATG CCATCCGTAA GATGCTTTTC TGTGACTGGT  
7251 GAGTACTCAA CCAACTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG  
7301 CTCTTGCCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT

7351 TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG  
7401 ATCTTACCGC TGTTGAGATC CAGTCGATG TAACCCACTC GTGCACCCAA  
7451 CTGATCTTCA GCATCTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA  
7501 CAGGAAGGCA AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT  
7551 TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA TTTATCAGGG  
7601 TTATTGTCTC ATGAGCGGAT ACATATTGA ATGTATTTAG AAAAATAAAC  
7651 AAATAGGGGT TCCGCGCACA TTTCCCGAA AAGTGCCACC TGACGTCGAC  
7701 GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGCGCCGGCT TCGAATAGCC  
7751 AGACTAACCT TTTTTTTAA TTTTATTTA TTTTATTTT GAGATGGAGT  
7801 TTGGCGCCGA TCTCCCGATC CCCTATGGTC GACTCTCAGT ACAATCTGCT  
7851 CTGATGCCGC ATAGTTAACG CAGTATCTGC TCCCTGCTTG TGTGTTGGAG  
7901 GTCGCTGAGT AGTGCAGCAG CAAAATTTAA GCTACAACAA GCCAAGGCTT  
7951 GACCGACAAT TGCAATGAAGA ATCTGCTTAG GGTTAGGCGT TTTGCGCTGC  
8001 TTGCG3ATGT ACGGGCCAGA TATACCGTGT GACATTGATT ATTGACTAGT  
8051 TATTAATAGT AATCAATTAC GGGGTCAATTA GTTCATAGCC CATATATGGA  
8101 GTTCCGCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA  
8151 ACGACCCCCG CCCATTGACG TCAATAATGA CGTATGTTCC CATACTAACG  
8201 CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGACTATT TACGGTAAAC  
8251 TGCCCACCTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA  
8301 TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG  
8351 ACCTTATGGG ACTTTCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC  
8401 TATTACCATG GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC  
8451 GGTTTGACTC ACGGGGATTG CCAAGTCTCC ACCCCATTGA CGTCAATGGG  
8501 AGTTTGTGTTT GGCACCAAAA TCAACGGGAC TTTCCAAAT GTCGTAACAA  
8551 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT  
8601 ATATAAGCAG AGCTCTCTGG CTAACTAGAG AACCCACTGC TTACTGGCTT  
8651 ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTT

FIGURE 18F

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FIGURE 19 A

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10 GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGACTCT 30 40 50 60  
 CCATGGTAA ATTTAACTAT AGAGGAATCC AGAGCTCAGA CTAAGATAACC GGTCAATCGA  
 70 TTGGAAATCT TGCGGCCGCT TGCTAGCACC AAGGGCCCAT CGGTCTTCCC 100 110 120  
 AACCTTAAGA AGCGCCGGGA ACGATCGGG TTCCCCGGTA GCCAGAAAGGG CCAGTTAGCT  
 130 TCCCTCCAAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT GCCTGGTCAA 150 160 170 180  
 AGGAGTCTT CGTGGAGACCC CGGGACCTCGC CGGGACCCGA CGGACCAAGT CCTGATGAAAG  
 190 CCCGAACCGG TGACGGTGTG GTGGAACTCA GGCGCCCTGA 210 220 230 240  
 GGGCTGGCC ACTGGCCACAG CACCTTGAGT CGGGGGACT GGTCGCCGA CGTGTGGAAAG  
 250 CCGGCCTGTC TACAGTCCTC AGGACTCTAC TCCCTCAGCA 270 280 290 300  
 GGGCGACAGG ATGTCAGGAG TCCCTGAGATG AGGGAGTCGT CGCACCAAGTG GCACGGGAGG  
 310 AGCAGCTTGG GCACCCAGAC CTACATCTGC AACGTGAATC 330 340 350 360  
 TCGTCGAACC CGTGGGTCTG GATGTAGACG TTGGACTTAG TGTTGGGTG GTGTGGTTC  
 370 GGGGACAAAGA AAGTTGGTGA GAGGCCAGCA CAGGGAGGA 390 400 410 420  
 CACCTGTTCTT TCAACCAACT CTCCGGTCTGT GTCCCTCCCT CCCACAGAGG CAACACCAAG  
 430 GCTCAGCGCT CTCGCTTGA CGCATCCGG CTAAGCAGCC 440 450 460 470 480  
 CGAGTGGCGA GGAACCGACCT GCGTAGGGCC GATACTCGG CCAGTCCAGG GCACCAAGGC  
 490 AGGCCCGTC TGCCTCTTCA CCCGGAGGCC TCTGCCCGC 500 510 520 530 540  
 TCCGGGGCAG AGGGAGAGT GGGCCCTCCGG AGACGGGGCC GGTCAAGTCCG CGTCGTTCCG  
 550 GGTCTTCTGG CTTCCTTCCC AGGCTCTGGG CAGGGCACAGG 560 570 580 590 600  
 CCAGACAGC GAAAAGGGG TCCGAGACCC GTCCTGTC GATCCACGGG GATTGGGTCC

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## pD17-hG1b

610                   620                   630                   640                   650                   660  
 CCCTGCACAC AAAGGGCAG GTCGCTGGCT CAGACCTGCC AAGAGCCATA TCCGGGAGGA  
 GGGACCTTGTC TTTCCCCGTC CACGACCGA GTCTGGACGG TCTCGGTAT AGGCCCTCCT  
 670                   680                   690                   700                   710                   720  
 CCCTGCCCT GACCTAAGCC CACCCCAAAG GCCAAACTCT CCACTCCCTC AGCTGGACA  
 GGGACGGGA CTGGATTCGG GTGGGTTC CGGTTTCAAGA GGTGAGGGAG TCGAGGCCTGT  
 730                   740                   750                   760                   770                   780  
 CCTTCTCTCC TCCAGATTC CAGTAACCC CAATCTTC'TC 'CTCGAGAGC CCAAATCTTG  
 GGAAGAGAGG AGGGCTTAAG GTCATTTGAGG GTPAGAAGAG AGACGTCCTCG GGTTTGAAC  
 790                   800                   810                   820                   830                   840  
 TGACAAACT CACACATGCC CACCGTCCC AGGTAAAGCCA GCCCAGGCC' CGCCCTCCAG  
 ACTGTTTGA CTGTTGTACGG GTGGCAGGG TCCATTTCGGT CGGGTCCGGGA GCGGGAGGTC  
 850                   860                   870                   880                   890                   900  
 CTCAAGCCGG GACAGGTGCC CTAGAGTAGC CTGCAATCCAG GGACAGGCC' CAGCCGGGTG  
 GAGTTCGGCC CTGTCCACGG GATCTCATCG GACCTPAGTC CCTGTCGGG GTCGGCCAC  
 910                   920                   930                   940                   950                   960  
 CTGACACGTC CACTCCATC TCTTCCTAG CACCTGAAC' 235' CCGTCAGTCT  
 GACTGTGAG GTGGAGGTAG AGAAGGAGTC GTGGACTTGA AG'AC'ACAG GAGCCCGCCCT' GGCAGTCAGA  
 970                   980                   990                   1000                   1010                   1020  
 'CCCTCTCCC CCCAAAACCC AGGACACCC TCATGATCTC 950 237' CCGTCACAT  
 AGGAGAAGGG GGGTTTGGG T'CCCTGTGGG AG'AC'ACAG GGCC'GGGGGA C'CCAGTGT  
 1030                   1040                   1050                   1060                   1070                   1080  
 GCGTGG'GGT CGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG TACCTGGAGC  
 AGGACACCCA CCTGCACTCG GTGCTTCTGG GACTCCAGTT CAAGTTGACC ATGACACTGC  
 1090                   1100                   1110                   1120                   1130                   1140  
 CCGTGGAGGT GCAATAATGCC AAGACAAAGC CGGGGGAGGA GCAGTACAC AGCACGTAC  
 CGCACCTCCA CGTATTACGG TCTCTTTCG GCGCCCTCC' CGTCATGTTG TCG'GCAATGG  
 1150                   1160                   1170                   1180                   1190                   1200  
 CTCTGG'CAG CGTCTCTACC GTCCCTGCC AGGACTGGGT GAAATGGCAAG 318' GAGTACAGT  
 CACACCACTC GCGGGAGTGG CACCACTGG TCCCTGACCCA C'ATACGGTC C'CTGCTGTC  
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FIGURE 19C.

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312	1210	1220	1230	1240	1250	1260
GCA	GGCTCTC	CAACAAAGCC	CTCCCCAGGCC	AACCATCTCC	AAAGCCAAAAG	
CCTTCCAGAG	GTTGTTTCGG	GAGGGTTCGG	CCATTCGAGAA	TTCGGTACGG	TTCGGTTC	
1270	1280	1290	1300	1310	1320	
GTGGACCCG	TGGGGTGCAG	GGGCCACATG	GACAGAGGCC	GGCTCGGCC	ACCTCTGCC	
CACCTGGGC	ACCCACCGCT	CCGGGTGTAC	CTGTCCTCCGG	CCGAGCCGGG	'GGGAGACGG	
1330	1340	1350	1360	1370	1380	
CTGAGAGTGA	CCGCTGTAC	AACCTCTGTC	CCTACAGGGC	AGCCCCGAGA	ACCACAGGTG	
GACTCTACT	GGCGACATGG	TTGGAGACAG	GGATCTTCGG	TGTTGGCTCT	TGGTGTCCAC	
1390	1400	1410	1420	1430	1440	
TACACCTGTC	CCCCATCCCG	GGATGAGGCTG	ACCAAGAAC	AGGTCAGGCCT	GACTCTGCC	
ATGTGGGACG	GGGGTAGGGC	CCTACTCGAC	TGGTCTCTGG	TCCAGTCGG	CTGACGGAC	
1450	1460	1470	1480	1490	1500	
GTCAAAGGCT	TCTATCCAG	CGACATGCC	GTGGAGTGGG	AGAGCAATGG	GCAGCCGGAG	
CAGTTCCGA	AGATGGGTC	GCTGTAGGG	CACTCTACCC	TCTCGTTPAC	CGTGGCCTC	
1510	1520	1530	1540	1550	1560	
AACAACATACA	AGACCAAGCC	TCCCCGTGCTG	GAOTCCGACG	GCTCCTTC	CCTCTACAGC	
TTCGTTGATGT	TCTGGTGGCGG	AGGGCACGAC	CTGAGGTGTC	CGAGGAAGAA	GGAGATGTCG	
1570	1580	1590	1600	1610	1620	
AAGCTCACCG	TGGACAAGAG	CAGGTGGCAG	CAGGGAAACG	TCTTCTCATG	CTCCGTGATG	
TTCGAGTGGC	ACCTGTTCTC	GTCCACCGTC	GTCCCTCTGC	AGAAGAGTAC	GAGGCACTAC	
1630	1640	1650	1660	1670	1680	
CATGAGGCTC	TGCAACACCA	CTACACGGAG	AAGAGCCCT	CCCTGTCTCC	GGGTAAATGA	
GTACTCCGAG	ACCTGTTGGT	GATGTGGTC	TTCCTGGAGA	GGGACAGAG	CCCATTTACT	
1690	1700	1710	1720	1730	1740	
GTGCGACGGC	CGGCAAGCCC	CGGCTCCCC	GGCTCTCGG	GTGCGACGG	GATGCTGGC	
CACGCTGCCG	GGCGTTCCGG	GGCGAGGGG	CCGAGAGGCC	CAGCGTGTCTC	CTACGAACCG	
1750	1760	1770	1780	1790	1800	
ACGTACCCCC	TGTACATACT	TCCCCGGGCC	CCAGCATGGA	ATTAAGCAC	CCAGCGCTGC	
TCCATGGGGG	ACATGTATGA	AGGGCCCGG	GGTGTGACG	TATTTCTGTC	GGTGGGACG	

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FIGURE 19D

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1810	1920	1830	1840	1850	1860
CCTGGCCCC GGACCCGGG	TGGGAGACT ACGGCTCTGAC	TGATGGTCT ACTACCAAGA	TTCCACGGGT AAGGTGCCC	CAGGCCAGT GTCGGGTCA	CTGAGGCCCTG GACTCCGGAC
1870	1880	1890	1900	1910	1920
AGTGGCA1GA TCACCGTACT	GGGAGGCAGA CCCTCCGTCT	GGGGTCCCC CGCCCCAGGGT	CTGTCCCCAC GACAGGGGTG	ACTGGCCAG TGACCGGGTC	GCTGTGCAGG CGACACGTCC
1910	1940	1950	1960	1970	1980
TGICCTGGG ACACGGACCC	CCCCCTAGGG GGGGATCC	1GGGGCTCAG ACCCCGAGTC	CCAGGGGCC GGTCCCCGAC	CCCTCGGCAG GGGAGCGTC	GTTGGGGAT CCACCCCTTA
1990	2000	2010	2020	2030	2040
TTGCCAGCGT AACGGTGGCA	GGCCCTCCCT CCGGGGAGGG	CCAGCAGCAC GGTCGTCGTG	CTGGCCCTGGG GACGGGACCC	CTGGGCCACG GACCCGGTGC	CGAACGCCCTA CTTCAGGGAT
2050	2060	2070	2080	2090	2100
GGAGCCCTG CCTCGGGAC	GGGACAGACA CCCTGTCGTG	CACAGCCCC GTGTCGGGA	GCCTCTGTAG CGGAGACATC	GAGACTGCC CTCTGACAGG	TGTCTGTGA ACAAGACACT
2110	2120	2130	2140	2150	2160
GGCCCCCTGT CGCGGGGACA	CCTCCCGACC GGAGGGCTGG	TCCATGCCA AGGTACGGGT	CTCGGGGCA GAGCCCCGGT	TGCTGGGGAT ACGACCCCTA	GCGCACCCGA CGACAGACAA
2170	2180	2190	2200	2210	2220
C'TA'GGC'YT GATACCGAAG	TGAGGGGGAA ACTCCGGCTT	AGAACCAAGCT TCTTGGTCGA	GGGGCTCTAG CCCCCAGATC	GGGGTATCCC CCCCATAGGG	TGTCTGTGA CGACAGACAA
2230	2240	2250	2260	2270	2280
GTAGGGCGC CATCGCCGGC	ATTAAGGGCG TAATTGCGGC	GGGGGTTGG CGCCCCAACCC	TGGTTAACGG ACCAAATGGC	CAGGTGACCC GTCGCACGG	CACGGGCCCT CGATGTGAAC
2290	2300	2310	2320	2330	2340
CCAGGCCCT GGTCGGGGGA	AGCGCCCGCT TCGGGGCGA	TCTTTCGCTT GGAAAGCGAA	TCTTCCCTTC AGAAAGGGAA	CTTCTCGCC GAAAGGGGG	ACGTTCGCC TGCAGGGGC
2350	2360	2370	2380	2390	2400
GCTTCGCCG CGAAACGGCC	TCAAGCTCTA AGTCGAGAT	TCCCTTTAGG TTAGCCCCGT	GTTCCGATTT AGTGCTTTAC	TTCCGATTT CAAGGAAATCC	TCAAGGAAATG

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2410      2420      2430      2440      2450      2460  
 CCCACCTCGA    CCCCAAAAAA    CTTGATTAGG    GTGATGGTTTC    ACGTAGTGGG    CCATCGCCCT  
 CCGTGGAGCT    GGGGTTTTTT    GAACTAATCC    CACTACCAAG    TGATCACC    GGTAGGGGA  
 2470      2480      2490      2500      2510      2520  
 GATAGACGGT    TTTTGGCCCT    TTGACGTTGG    AGTCACAGT    CTTAAATAGT    GGACTCTGT  
 CTATCTGCCA    AAAAGGGGA    AACTGCAACC    TCAGGTGCAA    GAAATTATCA    CCTCGAGAAC  
 2530      2540      2550      2560      2570      2580  
 'CCAAAC'GG    AACAAACACTC    AACCTATCT    CGGTCTATTC    'TTTGATTAA    TAAGGGATT  
 AGTTTGACCC    TTGTTGAG    TTGGGATAGA    GCCAGATAAG    AAAACTRAAT    ATTCCTTAA  
 2590      2600      2610      2620      2630      2640  
 TGGGGATTTC    GGCCTATTGG    TTTAAAAAATG    AGCTGATTAA    ACAAAATTTT    AACGGGAATT  
 ACCCTTAAG    CCGGATAACC    AATTTTTAC    TCGACTAAAT    TGTTTTTAAA    TTGCGCTTAA  
 2650      2660      2670      2680      2690      2700  
 AATCTGTGG    AATGTGTGTC    AGTTAGGGTG    TGGAAAGTCC    CCAGGCTCCC    CAGGCAGGCA  
 TTAAAGACCC    TTACACACAG    TCAATCCAC    ACCTTTCAGG    GGTCCCGAGGG    GTCCCGTCCGT  
 2710      2720      2730      2740      2750      2760  
 GAAGTATCCA    AAGCATGCAT    CTTCAATTAGT    CAGCAACCAT    AGTCCCGGCC    CTAACTCCGC  
 CTTCATACGT    TTGTACAGCA    GAGTTAACCA    GTCGTTGGTA    TCAAGGGGGGG    GATTGAGGGCG  
 2770      2780      2790      2800      2810      2820  
 CCATCCGCC    CCTAACTCCG    CCCAGTTCG    CCCATTCTCC    GCCCCATGGC    TGACTTAATT  
 GGTAGGGCGG    GGATGTAGGC    GGGTCAAGGC    GGGTAAGAGG    CGGGGTACCG    ACTGATTAAA  
 2830      2840      2850      2860      2870      2880  
 TTTTATTATA    TGAGGAGGCC    GAGGGCGCC    CGGCCTCTGA    GCTATTCCAG    AAGTAGTGAG  
 AAAATAAAAT    ACGTCTCCGG    CTCCGGGGGA    GCCGGAGACT    CGATAAGGTC    TTCACTCACTC  
 2890      2900      2910      2920      2930      2940  
 GAGGCTTTTT    TGGAGGCCATA    GGCTTTGCA    AAAAGCTTGG    ACAGCTCAGG    GCTGGGATT  
 CTCCGAAAAAA    ACCTCCGGAT    CCGAAAACGT    TTTTCGAACC    TGTGAGTCC    CGACGCTAAA  
 2950      2960      2970      2980      2990      3000  
 CGGCCAAAC    TTGACGGCAA    TCCTAGGGTG    AAGGCTGGTA    GGATTTTATC    CCCGGCTGCCA  
 CGGCCGTTTIC    AACCTGGGTIC    AGATCGCAC    TTCCGACCAT    CCTAAAATG    GCGCGACGGT

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3010 TCATGCTTCG ACCATTGAAAC 3020 TGCATGTCG 3030 CCGTGTCCCA 3040 AAATAATGGGG 3050 ATGGCAAGA  
 AGTACCAAGC TGGTAACCTG ACGTAGCAGC 3060 GGCACAGGGT TTTATACCCC TAACCGGTCT  
 3070 ACGGAGACCT ACCCTGGCC 3080 CCGCTCAGGA 3090 ACGAGTTCA 3100 GTRACTCCAA 3110 AGAATGACCA  
 TGCCTCTGGG 3120 TGGGACCGGA GGGGAGTCCT  
 3130 AACCTCTTC AGTGGAAAGGT 3140 AAACAGAATC 3150 TGGTGATTAT 3160 GCGTAGGAA 3170 ACCTGGTTCT  
 GTTGGAGAAG 3180 TCACCTTCCA TTGTGCTTAG ACCACTTATAA CCCATCCTT TGGACCAAGA  
 3190 CCATTCCTGA GAGAATTCGA 3200 CCTTTAAAGG 3210 ACAGAATTAA 3220 TATAGTTCTC 3230 AGTAGAGAAC  
 GGTAAAGGACT 3240 CTTCTTAGGT GGAATTTCCTCC 3250 GCTCATTTTC TGGCAAAAG 3260 TTGGATGAT 3270 GCCTTAAGAC  
 TCAAAGAACC ACCACGAGGA 3270 GCTCATTTTC 3280 3290 3300  
 AGTTCTTGG TGGTCTCTCT 3290 CGAGTAAAG AACGGTTTC AACCTACTA CGGAATTCTG  
 3310 TTATTGAACA ACCGGAAATG 3320 GCAAGTAAG 3330 3340 3350 3360  
 AATAACTTGT TGGCCTTAAC CGTCTATTC ATCTGTACCA AACCTATCG CCTCCGTCAA  
 3370 CTGTTTACCA CGAAGCCATG 3380 AATCAACCAAG 3390 3400 3410 3420  
 GACAATGGG! CTTGGTAC TTAGTTGGTC CGGTGAA!TC TGAGAAACAC TGTTCCCTAGT  
 3430 TGCAGGAATT TGAAGTGTAC 3440 ACGTGTTTC 3450 CAGAAATTGA 3460 3470 3480  
 ACGTCTTAA ACTTTCACG TGCAAAAGG GTCTTAACT AAACCCCTT ATATTTGAG  
 3490 TCCCAGAATA CCCAGGGGTG 3500 3510 3520 3530 3540  
 AGGGTC!PAT GGGTCCGGAG GAGAGACTCC AGGTCC!CTC TTTCCGTAG TTCATATTCA  
 3550 TTGAAGTCTA CGAGAAGAAA GACTAACAGG 3560 3570 3580 3590 3600  
 AACATCAG!T GCTCTCTCTT CTGATTGTCC TTCTACGAAA GTTCAGAGA CGGGGGAGG

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FIGURE 19G

## pD17-hG1b

3610 TAAGGCTATG CATTTTTATA 3620 AGACCATGGG 3630 ACTTTTGGTG 3640 GCTTTAGATC 3650 TCTTGTGAG 3660  
 ATTTGATAC GTAAAAAATAT TCTGGTACCC CGAAAACGAC CGAAATCTAG AGAAACACTT  
 3670 GGAACCTTAC TTCTGTGGTG 3680 TGACATTAATT 3690 3700 CCTACAGAGA 3710 TTAAAGCTC  
 CCTTGGAAATG AAGACACCAC ACTGTATTAAC CCTGTTTGTAT GGATGTCTCT AAATTCGAG  
 3720  
 TAACGGAAAT ATAAATTTT TAAGTGTATA 3730 3740 3750 3760 3770 3780  
 ATTCCATTTA TATTAAATTAA ATTCAACATAT TACACAAATT' GATGACTAAG ATTAACAAAC  
 TGTATTGTAG ATTCACACT ATGGAACTGA 3790 3800 3810 3820 3830 3840  
 ACATAAAATC TAGGTGGA TACCTGACT ACTTACCCCTC GTCACCAACCT TAGGAAATT  
 3850 TGAGGAAAC CTGTTTGTCT 3860 3870 3880 3890 3900  
 ACTCCTTTG GACAAACGA GTCTCTTTA CCGTAGATCA CTACTACTC GATGACGACT  
 3910 CTCTCAACAT TCTACTCCTC 3920 3930 3940 3950 3960  
 GAGAGTTGTA AGATGAGGAG 3970 3980 3990 4000 4010 4020  
 'TCAGAAATG CTAAGTTTT TGAGTGTG 4030 4040 4050 4060 4070 4080  
 AAGTCTAAC GATTCAAAA ACTCAGTACG 4090 4100 4110 4120 4130 4140  
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 4150 TCCACACAGG CATTAGGTGT 4160 4170 4180 4190 4200  
 AGGTGTGTCG 41  
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FIGURE 19H

## pD17-hG1b

4210 4220 4230 4240 4250 4260  
 CTTTTAATT TGTAAAGGGG TAAATAAGGA ATATTGATG TATAGTCCT TGACTAGAGA  
 GAAAATAA ACATTTCCCC RATTATCCT TATAAACTAC ATATCACGGA ACTGATCTCT  
  
 4270 4280 4290 4300 4310 4320  
 TCAATAATCG CCATACCACA TTTGTAGAGG TTTTACCTGC TTTAAAAAC CTCCACACCC  
 AGTATAGTC GGTATGGT AAACATCTCC AAAATGAACG AAATTTTTCG GACGGGTGCG  
  
 4330 4340 4350 4360 4370 4380  
 TCCCCCTGAA CCTGAAACAT AAAATGAAATG CAATTGTTGT TGTTAACCTTG TTATTGCGAG  
 AGGGGACTT GGACTTTGTA TTTTACTTAC GTAAACAAACA ACAATTGAAC AAATAACGTC  
  
 4390 4400 4410 4420 4430 4440  
 CTTATAATGG TTACAAATAA AGCAAAATAGCA TCACAAATT CACAAATAAA GCATTTTTT  
 GAATATTACC AATGTTTATT CGTTTATCGT AGTGTTTAAA GTGTTTATT CGTAAAAAAA  
  
 4450 4460 4470 4480 4490 4500  
 CACTGCATTG TAGTTGGTGTGTT TTGTCACAAAC TCATCAATTG ATCTTATCAT GTCTGGATCG  
 GTGACCTAAG ATCAAACACCA AACAGGTTTG AGTAGTTACA TAGAATAGTA CAGACCTAGC  
  
 4510 4520 4530 4540 4550 4560  
 GCTGGATGAT CCTCCAGGCC GGGGATCTCA TGCTGGAGT CTTCGCCAC CCCAACCTGT  
 CGACCTACTA GGAGGTGCGC CCCCTAGAGT AGCACCTCAA GAAGGGGTG GGGTTGAACA  
  
 4570 4580 4590 4600 4610 4620  
 TTATTCGAGC TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTG ACAAAATAAG  
 AATAACGTC AATATTACCA ATGTTTATT CGTTTATCGTA GTGTTAAAG TGTTTATTC  
  
 4630 4640 4650 4660 4670 4680  
 CAAATTTTTC ACTGCCATTCT AGTTGGGGTT TGTCACAACT CATCAATGTA TCTTATCATG  
 GTAAAAAAAG TGACGTAAGA TCAACACCAA ACAGGTTGAGA GTAGTTACAT AGAATAGTAC  
  
 4690 4700 4710 4720 4730 4740  
 TCTGTTATACC GTCGACCTCT AGCTAGAGCT TGCGTAAATC ATGGTCAATAG CTTGTTCCCTG  
 AGACATATGG CAGCTGGAGA TCGATCTCGA ACCGCATTAG TACCAAGTAC GACAAAGGAC  
  
 4750 4760 4770 4780 4790 4800  
 TGTGAATTG TTATCCGGTC ACATTTCCAC ACAAACATACG AGCGGAAAGC ATAAAGTGTAA  
 ACATTTAAC ATAGGGCGAG TCTTAAAGGTG TGTTCATGCC TCGGCCTTCG TAAATCACAT

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FIGURE 19I

pD17-hG1b

AAGCC'GGGG	4810	4820	4830	4840	4850	4860
T'CGGACCCC	ACGGATTACT	CACTCGATTG	AGTGTAAATT	'GCCGTTGCC	TCACTGCCCG	
CT'CCAGTC	GGGAAACCTG	TCGNGCAGC	TGCCATTAAATG	ACGCCAAGGGG	AGTGACGGGC	
GAAAGGTCAAG	CCCTTGAC	AGCACGGTCG	ACGTAATTAC	T'PAGGCCGTT	GCGGGCCCT	
4930	4940	4950	4960	4970	4980	
GACGGGT'rr	GGGTATTGGG	CGCTCT'CCG	CTTCCT'CGCT	CACTGACTCG	CTCGGCTCGG	
CTCCGCCAAA	CGCATAACCC	GCGAGAAGGC	GAAGGAGCGA	GTGACTGAGC	GACGCCAGCC	
4990	5000	5010	5020	5030	5040	
TCGTTGGCT	GCGGGAGGG	GTATCAGCTC	ACTCAAAAGGC	GGTAATACGG	TTATCCACAG	
AGCAAGCCGA	CGCCGCTCGC	CATAGTCGAG	TGAGTTTCGG	CCATTATGCC	AATAGGTGTC	
5050	5060	5070	5080	5090	5100	
AATCAGGGGA	TAACGCGAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	
TTAGTCCCT	ATTCGCTCCT	TTCTTGATCA	CTCGTTTCC	GGTCGTTTTC	CGGTCTTGG	
5110	5120	5130	5140	5150	5160	
GTAAAAGGC	CGCGTTGCTG	GGGTTTTC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	
CATTTTCCG	GGCCAACGAC	CGCAAAAGG	TATCCGGAGG	GGGGGAACTG	CTCGTAGTGT	
5170	5180	5190	5200	5210	5220	
AAATTCAGCG	C'TCAAGTCAG	AGGTGGGAA	ACCCGACAGG	ACTATAAGA	TACCAAGCGT	
TTT'AGCTUC	GAATTCAGTC	TCCACCGCTT	TGGCTGTC	TGATATTC'	ATGTCCTCGCA	
5230	5240	5250	5260	5270	5280	
T'TCCCTGGA	AAAGCTCCCTC	GTGGCGCTCTC	CTGTTCCGAC	CCTGGCGCTT	ACCGGATACC	
AAGGGGACCC	T'TCGAGGGAG	CACGGAGAG	GACAAGGCTG	GGACGGGGAA	TGGCTATGG	
5290	5300	5310	5320	5330	5340	
TGTCCGCCCT	TCTCCCTTCG	GGAAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	
ACAGGGGGAA	AGAGGGAAAGC	CCTTCGACCC	GCGAAAGAGT	TACGAGTGG	ACATCCATAG	
5350	5360	5370	5380	5390	5400	
TCACTTGCGT	GTAGGTCGTT	CGCTCCAAAGC	TGGGCTGTTG	GCACGAAACC	CCCGTTCAAG	
AGTCAGGCCA	CATCCAGCAA	GGCAGGGTCG	ACCCGACACA	CGTGCTTGGG	GGCCAAGTCG	

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FIGURE 19J

pD17-hG1b

5410	5420	5430	5440	5450	5460
CCGACCCGCTG	CGCCTTATCC	GGTAACATAC	GTCTGAGTC	CAACCCGGTA	AGACACGACT
GGCTGGGAC	GGGAAATAGG	CCATTGATAG	CAGAACTCAG	GTGGGGCCT	TCTTGCTGTA
5470	5480	5490	5500	5510	5520
TATGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGGGGTG
ATAGGGTGA	CCGTGTCGG	TGACCATGTT	CCTAAATCGTC	TCGCTCCATA	CATCCGCCAC
5530	5540	5550	5560	5570	5580
CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTGGTA
GATGTC'CAA	GAACCTCACC	ACCGGATGTA	TGCCGATGTT	ATCTTCTGT	CATAAACCAT
5590	5600	5610	5620	5630	5640
TCTGGCTCT	GCTGAAGCCA	GTACCTCTG	GGAAAAGGT	TGGTAGCTCT	TGATCCGGCA
AGACGGAGA	CGACCTCGGT	CAATGGAAGC	CTTTTCTCA	ACCATCGAGA	ACTAGCCGT
5650	5660	5670	5680	5690	5700
AACAAACAC	CGCTGGTAGC	GGTGGTTTT	TGTTTGGCAA	GCAGCGAGATT	ACGGCGAGAA
TGTTGGTG	GGGACCATCG	CCACCAAAA	AACAAACGTT	CCTCGTCTAA	TGCGGTCTT
5710	5720	5730	5740	5750	5760
AAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTCTACGGG	GTCTGAGGCT	CAGTGGAAACG
T'RTT'CCATG	AGTCTCTCA	GGRAACTAGA	AAAGATGCC	CAGACTGCGA	GTCAACCTTGC
5770	5780	5790	5800	5810	5820
AAAACTCACT	TAAAGGGATT	TGGTCATGA	GATTATCAA	AAGGATCTTC	ACCTAGATCC
T'RTTGACTGC	AAATCCCTAA	AACCAGTACT	CTAATAGCTT	T'CCCTAGAAG	TGGATCTAGG
5830	5840	5850	5860	5870	5880
TTTAAATTA	AAAATGAAGT	TTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG
AAAATTAAT	T'TTTACTCTCA	AAATTTAGTT	AGATTTCTAA	TATACTCTTT	TGAACCAAGAC
5890	5900	5910	5920	5930	5940
ACAGTACCA	ATGCTTAATC	AGTGGGCCAC	CTATCTCAGC	GATCTCTCTA	TTTCGTTCAT
TGTCAATGGT	TAGGAATTAG	TCACTCCGTG	GATAGACTCG	CTAGACAGAT	AAAGCAAGTA
5950	5960	5970	5980	5990	6000
CCATAGTTCG	CTGACTCCCC	GTCTGTTAGA	TAACCTACGAT	ACGGGAGGGC	TTACCATCTG
CC'PATC'ACC	GACTGAGGGG	CAGCACATCT	ATTGATGCC'A	1GCCCTCCCG	AAATGGTAGAC

4315.

FIGURE 19K

pD17-hG1b

GCCCCAGTGC	TGCAATGATA	CCGGAGACC	CACGCCYACCC	GGCTCCAGAT	TTATCAGCAA	6060
CGGGGTACCG	ACGTTTACTAT	GGGGCTCTGG	GTGGCAGTGG	CCGAGGTCTA	AATAGTCCGT	
6070	6080	6090	6100	6110	6120	
TAAACCAGCC	AGCCGGAAAG	GCCGAGGCCA	GAAGTGGTCC	TGCAACTTAA	TCCGCCCTCCA	
ATTTGGCGG	TGGGCCCTCC	GGGCTCGGT	CTTCACCAGG	ACGTTGAAT	ACCCGGAGGT	
6130	6140	6150	6160	6170	6180	
TCCAGTCTAT	TAATTGGTTC	CGGGAAAGCTA	GAGTAAGTAG	TTCGCCAGTT	AATAGTPTMGC	
AGGTCAAGATA	ATTAACAAACG	GCCCTTCGAT	CTCATTCATC	AAGGGTCAA	TTATCAAACG	
6190	6200	6210	6220	6230	6240	
GCAACGTTGT	TGCCCATGGCT	ACAGGCATCG	TGGTGTCAAG	CTCGTCTGTT	GGTATGGCTT	
CGTTGCAACA	ACGGTAACGA	TGTCCGTAGC	ACCACAGTGC	GAGCAGCAA	CCATACCGAA	
6250	6260	6270	6280	6290	6300	
CATTAGGCTC	CGGTTCCTCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTCTGCAAAA	
GTAACTGAG	GCCAAAGGGTT	GCTAGTTCGG	CTCAATGTAC	TAGGGGGTAC	AACACGTTT	
6310	6320	6330	6340	6350	6360	
AAGGGGTTAG	CTCCCTTCGGT	CCTCCGATCG	TGTCAGAAG	TAAGTTGGCC	GCAGTGTAT	
TCGGCCAAATC	GAGGAAGCCA	GGGGCTAGC	AAACAGTCTTC	ATTCAACCGG	CGTCACAAATA	
6370	6380	6390	6400	6410	6420	
CACTCATGGT	TATGGCAGCA	CTGCATATT	CTCTTACTGT	CATGCCATCC	GTPAGATGCT	
GTGAGTACCA	ATACCGTCGT	GACGTATTA	GAGAATGACA	GTPACGGTAGG	CATTCCTACGA	
6430	6440	6450	6460	6470	6480	
TTCCTCTGAC	TGGTGAAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGCGGACCGA	
AAAGACACTG	ACCACTCATG	AGTGGTTCA	GTAAAGCTCT	TATCACATAC	GCCGCTGGCT	
6490	6500	6510	6520	6530	6540	
GTGCTCTTGT	CCGGCGTCA	ATACGGATA	ATACCGGCC	ACATAGCAGA	ACTTTAAAG	
CAACGAGAAC	GGGGCGCAAGT	TATGCCCTAT	TATGGCGGG	TGTATGCT	TGAAATTTC	
6550	6560	6570	6580	6590	6600	
TGCTCATCAT	TGAAAACGTT	TCTTCGGGGC	GAAAACCTCTC	AAGGATCTTA	CCGCTGTTGA	
ACCGAGTACCA	ACCTTTTGGCA	AGAAGCCCCG	CTTGTGAAG	TICCTAGAAAT	GGCGACAACT	

FIGURE 19L

## pD17-hG1b

6610	6620	6630	6640	6650	6660
GATCCAGTC GATGTAACCC	ACTCGTCCAC	CCAACTGTCAC	TTCAGGATCT	TTCAGCTTC	TTCAGCTTC
CTAGGTCAAG CTACATGGG	TGAGCACCGT	GGTTGACTAG	AAGTCGTAG	AATGAAAGT	
6670	6680	6690	6700	6710	6720
CCAGCGTTTC TGGCTGAGCA	AAAACGGAA	GGCAAAATGC	CGCAAAAG	GGAAATRAGGG	
GGTCGCAAAG ACCCACTCGT	TTTTCCTCCCT	CCGTTTTAGC	GGGTTTTTC	CCTTATTCCC	
6730	6740	6750	6760	6770	6780
CGACACGGAA ATGTTGAATA	CTCATACTCT	TCTCTTTTCA	ATATTATGAG	AGCATTATTC	
GCTGCGCTT TACAACTT	GAATGAGA	AGGAAAAAGT	TATAATACT	TCTGAAATAG	
6790	6800	6810	6820	6830	6840
AGGGTTATTG TCTCATGAGC	GGATACATATA	TTCGAATGTT	TTAGAATAAT	AAACAATAAG	
TCCCCAATAAC AGAGTACTCG	CCTATGTATA	ACTTACATA	AACTCTTTA	TTTGTTTATC	
6850	6860	6870	6880	6890	6900
GGGTTCGGG CACATTCCC	CGAAAAGTGC	CACCTGACGT	CGACGGATCG	GGAGATCTGC	
CCCAGGGCGC G'GTAAAGG	GCCTTTCACG	GTGGACTGCA	GCTGCCATGC	CCTCTAGACCG	
6910	6920	6930	6940	6950	6960
TAGGTGACCT GAGGGGGGCC	GGCTTTCGAAT	AGCCAGAGTA	ACCTTTTTT	TTAAATTAT	
ATCCACTGGA CTCCGGCGG	CCGAAGCTTA	TGGTCTCAT	TGCAAAAAAA	ATTTAAATAA	
6970	6980	6990	7000	7010	7020
T'TTATTTCAT TTTTGAGATG	GAGTTGGCG	CCGATCTCCC	GATCCCCAT	GGTCGACTCT	
AATAAAATA AAAACTCTAC	CTCAAACCGC	GGCTAGAGGG	CTAGGGATA	CCAGCTGAGA	
7030	7040	7050	7060	7070	7080
CACTACAATC TGCTCTGATG	CCGCATAGTT	AAGCCAGTAT	CTGGCTCCCTG	CTTGAGTGT	
GTCATGTTAG ACAGACTAC	GGCGTATCAA	TTCGGTCATA	GACGGGGAC	GAACACACAA	
7090	7100	7110	7120	7130	7140
GGAGGTGGCT GAGTAGTGGG	CGAGCAAAAT	TTAAGCTACA	ACAAGGCAAG	GCTTGACCGA	
CCTCCAGCGGA CTCATCACCG	GCTGGTTTA	AATTCTGATGT	TGTTTCGTTTC	CGAACATGGCT	
7150	7160	7170	7180	7190	7200
CAATGCCATG AGAAATCTGC	TTAGGGTTAG	GGGTTTTGG	CTGCTCTGGC	ATGTAACGGGC	
GTTAACGTAC TCTTGTAGAC	AATCCCAATC	CGCAAAACCG	GACGAAGGCC	TACATGCCCG	

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## pD17-hG1b

7210 7220 7230 7240 7250 7260  
 CAGATATACTG CGTTGACATT GATTATGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC  
 GTCTATATGC GCAACTGTAA CTAATACTG ATCAATAATT A1CATTTAGTT AATGCCCGAG  
  
 7270 7280 7290 7300 7310 7320  
 ATTAGTCACT AGCCCATATA TGGAGTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC  
 TAATCAAGTA TCGGGTATAT ACCTCAAGGC GCATGTATT GAATGCCATT TACGGGGCGG  
  
 7330 7340 7350 7360 7370 7380  
 TGGCTGACCG CCCAACGACC CCCGCCATT GACGTCAATA ATGACGTATG T1CCCATAGT  
 ACCGAC1GGC GGGPTGCTGG GGGGGGTAA CTGGCACTATPACTGCACTAC AAGGGTATCA  
  
 7390 7400 7410 7420 7430 7440  
 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAC TATTACGGT AAACCTGCCA  
 T1GCGGTTAT CCCTGAAAGG TAACGTCACT TACCCACCTG ATAAATGCCA TTGACGGGT  
  
 7450 7460 7470 7480 7490 7500  
 CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCC CCTATTGACG TCAATGACGG  
 AACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCCGG AGTACTGCG  
  
 7510 7520 7530 7540 7550 7560  
 TAAATGGCCCG CCCTGGCATT ATGCCCAAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA  
 ATTACCGGG CGGACCGTAA TACGGGTCAAT GTACTGGAAT ACCCTGAAAG GATGAACCGT  
  
 7570 7580 7590 7600 7610 7620  
 GTACATCTAC GTATTAGTCA TCGCTTATTAC CATGGTGTATG CGGTTTGGC AGTACATCAA  
 CATGTAGATC CATAATCAGT AGCGATTAATG GTACCACTAC GCCAAACCG TCATGTAGTT  
  
 7630 7640 7650 7660 7670 7680  
 TGGGCGTGGAA TAGGGGTTTG ACTCACGGGG ATTCCAAGT CTCCACCCCA TTGACGTCAA  
 ACCCGCACCT ATGGCCAAAC TGAGTGGCCC TAAAGGTCA GAGGTGGGT AACTGCAGTT  
  
 7690 7700 7710 7720 7730 7740  
 TGGGAGTTTG TTTGGCAC AAAATCAACG GCACATTCCA AAATCTCGTA ACAACTCCGC  
 ACCCTCAAC AAAACCGTGG TTTAGTTGC CCTGAAAGGT TTACAGCAT TGTGAGGGC  
  
 7750 7760 7770 7780 7790 7800  
 CCCATTGACG CAAATGGGG GTAGGGCTGT ACCGGGGAG GTCTATATAA GCAGAGGCTCT  
 GGGTAACCTGC GTTACCGGC CATCCGCACA TGCACCCCTC CAGATATATT CGTCCTCGAGA

46 156

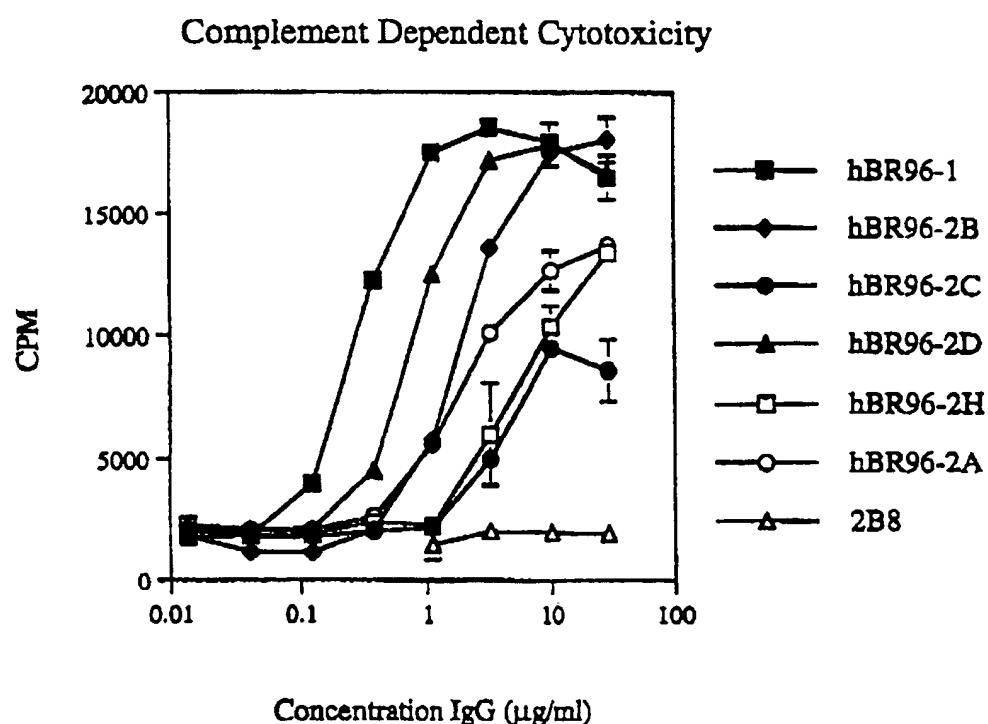
FIGURE 19N

## pD17-hG1b

7810 7820 7830 7840 7850 7860  
CTGGCTTAAC AGAGAAACCC A CTGCTTACTG GCTTATCGAA ATTAAATCGA CTCACTATAG  
GACC GATTGA TCTCTGGGT GACGAATGAC CGAATAGCT TAATTATGCT GAGTGATATC  
7870 7880  
GGAGACCCAA GCTT CCTCTGGGT CGAA

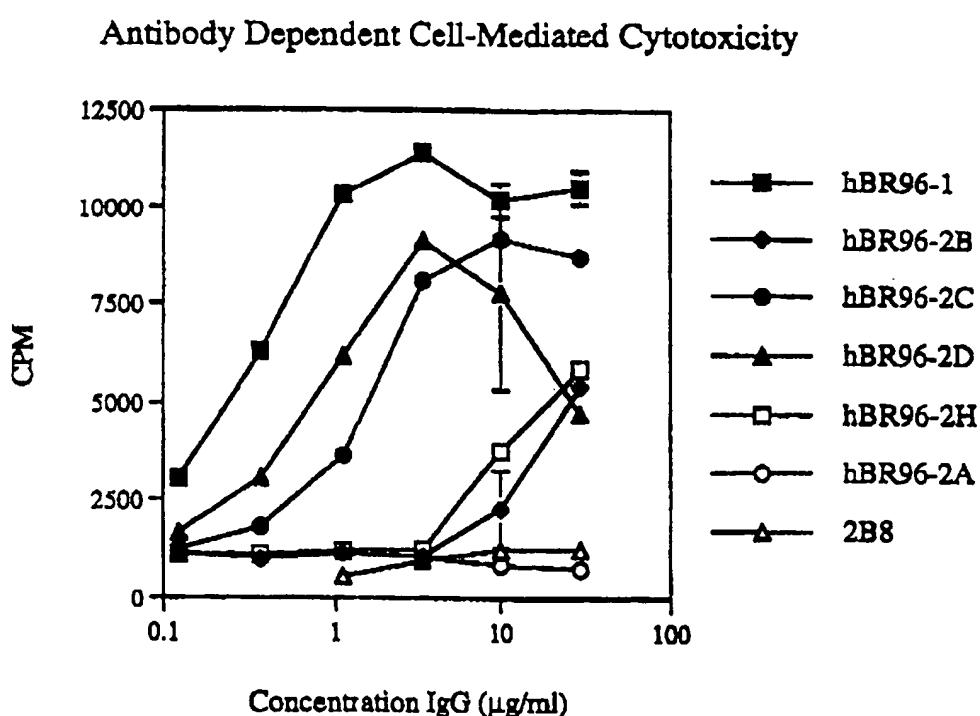
47156

FIGURE 20



4815

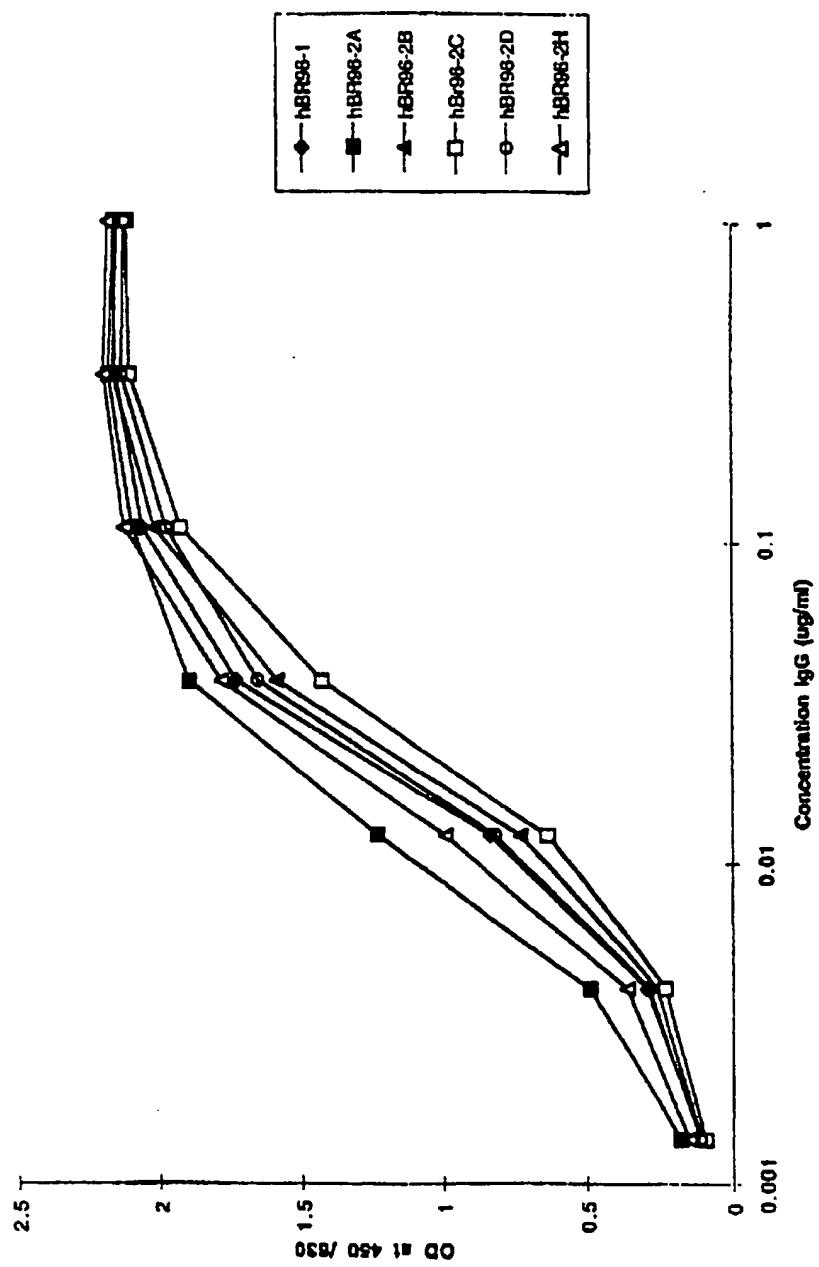
FIGURE 21



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FIGURE 22

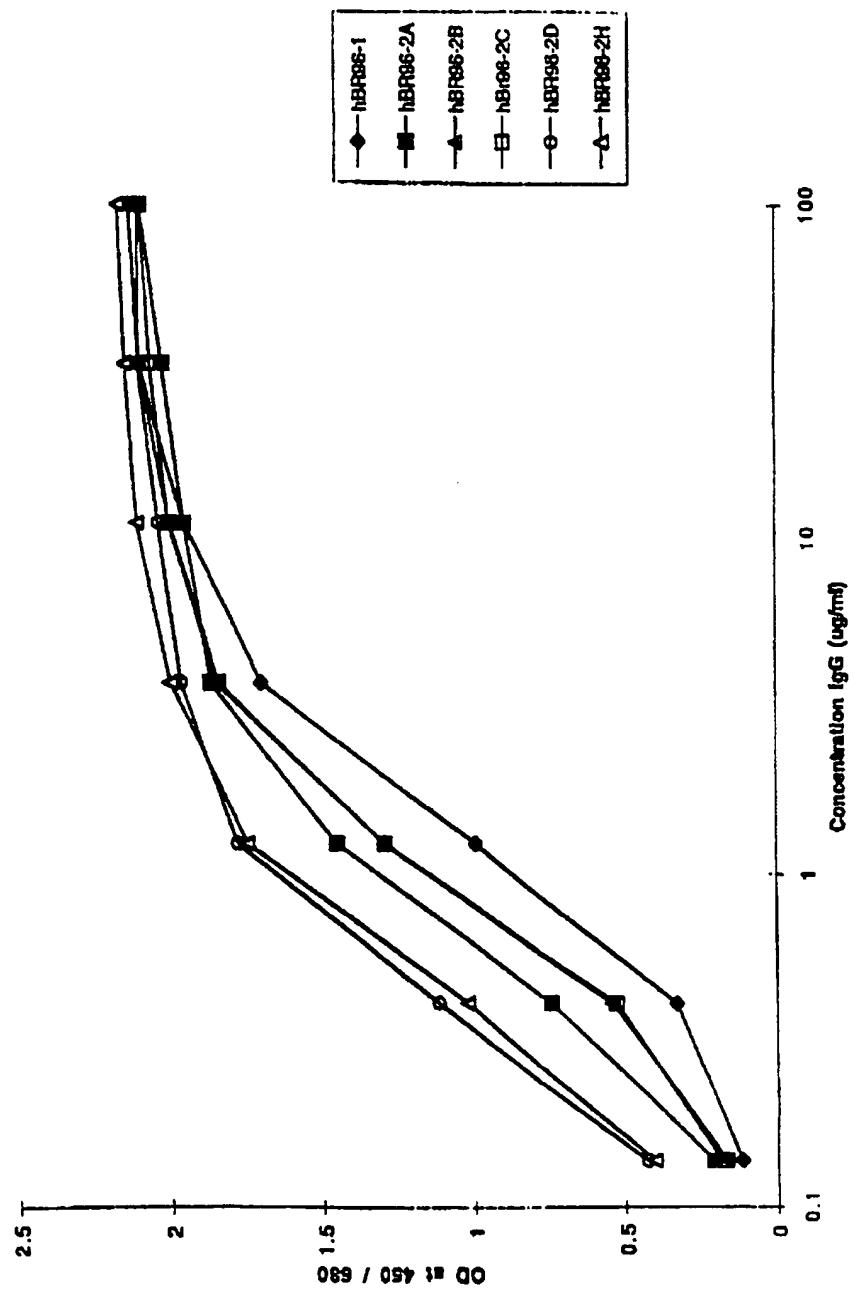
## Binding activity of hBR96-2 constant region mutants on LeY-HSA



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FIGURE 23

## Binding activity of hBR96-2 constant region mutants on LNFPIII-BSA



51156

Figure 24

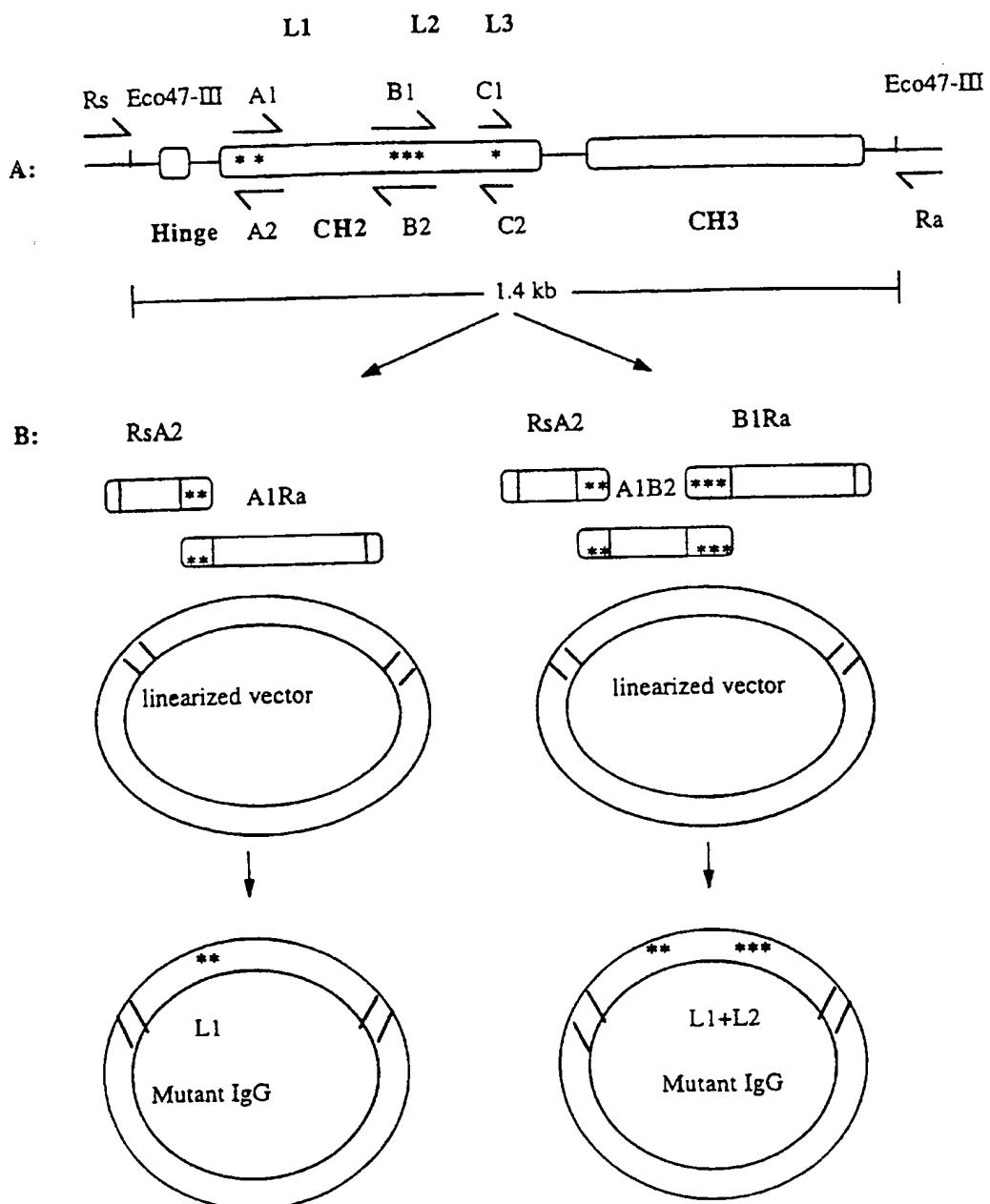
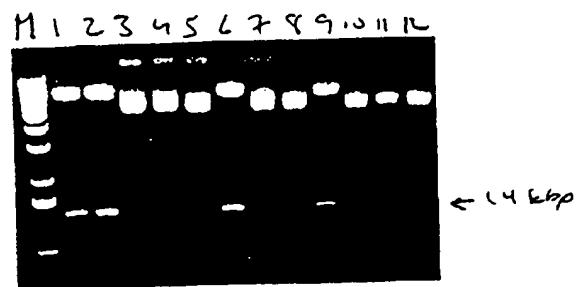


Figure 25



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Figure 26

## hBR96-2 Heavy Chain Variable Region (VH)

1                    11                    21                    31                    41  
 EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYYMYWVRQA PGKGLEWVSY  
 51                    61                    71                    81                    91  
 ISQDGDIRDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL  
 101                    111  
 ADGAWFAYWG QGTLVTVSS

## human IgG1 constant

1                    21                    31                    41  
 STKGPSVFPL APSSKSTSGG TAALGCLVKD  
 YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTY PSSSLCTQTY  
 ICNVNPKPSN TKVDKKVEPK SCDKTHTCPP C<sup>104</sup>PEI<sup>115</sup>Q<sup>116</sup> SVFLFPPKPK  
 DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS  
 318 320 322 324 326 328 329 330 331 332 333  
 TYRIVSVLTV LHQDWLNGK<sup>318</sup> Y<sup>320</sup>IVSNKAL P<sup>322</sup>EKTISK<sup>324</sup> AKGQPREPQV<sup>326</sup>  
 YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL  
 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

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## Figure 27

## hBR96-2A: Heavy Chain Variable Region (VH)

1 11 21 31 41  
EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYMYMYWVRQA PGKGLEWVSY  
51 61 71 81 91  
ISQDGDDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL  
101 111  
ADGAWFAYWG QGTLVTVSS

## hBR96-2A: Human Heavy Chain IgG1 Constant Region ΔCH2

A STKGPSVPPA PSSSKSTSCG DAALGCLVKD YFPEPVTVSW NSGALTSGVH  
TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK  
SCDKTHTCPP CP CQPREPVY YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA  
VEWESNGQPE NNYKTPPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM  
HEALHNHYTQ KSLSLSPGK

## Figure 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

VH  
1 EVNLVESGGG LVQPGGSLKV SCVTSGPTPS DYYMYWVRQT PEKRLEWVAY  
51 ISQGGDITDY PDTVKGRFTI SRDNAKNTLY LQMSRLKSED TAMYYCARGL  
101 DDGAWFAYWG QGTLVTVSWA STKGPSVFPL APSSKSTSGG TAALGCLVKD  
151 YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY  
201 ICNVNHKPSN TKVDDKKVEPK SCDKTHTCPP C<sub>61</sub>QPREPOV YTLPPSRDEL  
251 TKNQVSLTCL VKGFYPSDIA VENESNGQPE NNYKTTPPVL DSDGSFFLYS  
301 KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

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# INTERNATIONAL SEARCH REPORT

Intern: AI Application No  
PCT/US 97/13562

A. CLASSIFICATION OF SUBJECT MATTER				
IPC 6	C12N15/62	A61K39/395	A61K38/17	A61K47/48
	C07K16/30	C07K16/46	C07K16/00	C12N15/13
	C12N5/10	//C07K19/00		C12N1/21

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>S. GILLIES ET AL.: "Antigen binding and biological activities of engineered mutant chimeric antibodies with human tumor specificities." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 1, no. 1, 1990, STONEHAM, MA, USA, pages 47-54, XP002050448 see the whole document</p> <p>---</p> <p>-/-</p>	1-8, 23-25

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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Date of the actual completion of the international search

Date of mailing of the international search report

17 December 1997

21.01.98

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## INTERNATIONAL SEARCH REPORT

Intern'l Application No  
PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. SCHREIBER ET AL.: "An unmodified anticarcinoma antibody, BR96, localizes to and inhibits the outgrowth of human tumors in nude mice." CANCER RESEARCH, vol. 52, no. 12, 15 June 1992, BALTIMORE, MD, USA, pages 3262-3266, XP002050449 see abstract	33,35,36
A	---	1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for C1q on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7, 8
A	J. LUND ET AL.: "Human Fc $\gamma$ RI and Fc $\gamma$ RII interact with distinct but overlapping sites on human IgG." THE JOURNAL OF IMMUNOLOGY, vol. 147, no. 8, 15 October 1991, BALTIMORE, MD, USA, pages 2657-2662, XP002050451 cited in the application see abstract	1,2,5,7, 8
A	Y. XU ET AL.: "Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 5, 4 February 1994, BALTIMORE, MD, USA, pages 3469-3474, XP002050452 cited in the application see abstract see discussion	1-8
A	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	1,2,5,7, 8
	---	-/-

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## INTERNATIONAL SEARCH REPORT

Internat'l Application No  
PCT/US 97/13562

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category <sup>2</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>L. TAN ET AL.: "Influence of the hinge region on complement activation, Clq binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document</p> <p>---</p> <p>EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application</p> <p>see examples</p> <p>see claims</p> <p>-----</p>	1-8
A		11-18, 23,25, 28,29, 31-52

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 97/13562

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/13562

### FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark : Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 97/13562

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 699756 A	06-03-96	AU 2834995 A CA 2155397 A JP 8191692 A	15-02-96 05-02-96 30-07-96

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